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(57) Abstract

The invention provides a composition useful as a pharmaceutical excipient, the method of producing same, and the pharmaceutical compositions obtained thereby. In particular, the invention has applicability to increasing the solubility of poorly soluble therapeutically active compounds, by means of an excipient comprising a mixture of: (a) saturated polyglycolyzed glycerides, and (b) polyoxypropylene–polyoxyethylene block copolymers, whereby the solubility of the poorly soluble therapeutically active compound in the pharmaceutical composition is enhanced.

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SOLID STATE SOLUTIONS AND DISPERSIONS OF POORLY WATER SOLUBLE DRUGS

Field of the Invention

This invention relates to a composition especially useful as a pharmaceutical solutibility excipient, the method of producing same, and the pharmaceutical compositions obtained thereby. In particular, the invention has applicability to increasing the solubility of poorly soluble therapeutically active compounds.

Summary of the Invention

The invention provides a pharmaceutical solubility enhancing excipient for a pharmaceutical composition comprising a poorly water soluble therapeutically active compound, said excipient comprising a mixture of:

(a) saturated or unsaturated polyglycolyzed glycerides, and

(b) polyoxypropylene-polyoxyethylene block copolymers,

The invention further provides a pharmaceutical composition comprising a solid state solution or solid state dispersion of a poorly water soluble therapeutically active compound and a pharmaceutically acceptable excipient, said excipient comprising a mixture of:

(a) saturated or unsaturated polyglycolyzed glycerides, and

(b) polyoxypropylene-polyoxyethylene block copolymers,

whereby the solubility of the poorly soluble therapeutically active compound in the pharmaceutical composition is enhanced.

The invention further provides methods for making the above compositions. In the first instance (a) and (b) are mixed to form an excipient mixture. In the second instance, to form a pharmaceutical composition, heating said polyglycolyzed glycerides and polyoxypropylene-polyoxyethylene block co-polymer sufficiently to melt the ingredients,

adding the therapeutic agent to the molten mixture of polyglycolyzed glycerides and polyoxypropylene-polyoxyethylene block co-polymer

maintaining the mixture at a sufficient temperature for a sufficient time to dissolve or disperse the pharmaceutical agent.

The method aspect of the invention further comprises adding one or more optional excipients, whereby:

(A) the solubility of the therapeutically-active compound in the polyglycolyzed glyceride:polyoxypropylene-polyoxyethylene block co-polymer mixture is increased, or

(B) the melting point of the non-drug components is set, whereby at least one melting point peak belonging to the non-drug components is present between 30-80°C in the final composition, when analyzed by thermal analytical techniques.

The method aspect of the invention further comprises

maintaining the resulting mixture in the molten form, with constant stirring to ensure homogenous distribution of the drug in the system, and then

subjecting the molten mixture to one or more of the following operations:

I) allowing the mixture to congeal to a solid mass, and then extruding the mixture through a hot melt extruder into a powder;

II) milling the mixture using equipment that would maintain the milling conditions at room temperature or below the melting point of the non-drug components of the system to enable milling the composition into a powder;

III) spray-congealing the mixture in a spray drier or fluidized bed drier to a powder;

IV) congealing the mixture onto one or more optional excipients in a spray drier, fluidized bed drier, rotor, high shear granulator, planetary mixer, blender, or any conventional food and pharmaceutical processing equipment;

V) formulating the above-mentioned powders in pharmaceutical tablets, capsules, powders for inhalation, suppositories, suspensions, and emulsions; or

VI) congealing said mixture onto a solid pharmaceutical tablet, capsule or granule.

It has been discovered that by utilizing a mixture of: (a) saturated or unsaturated polyglycolyzed glycerides, as exemplified by the commercial Gelucire compositions and (b) polyoxypropylene-polyoxyethylene block copolymers, as exemplified by the commercial Pluronic surfactants, it is possible to prepare solid state solutions or solid state dispersions containing poorly soluble therapeutically active compounds, which compositions, in turn, provide a high degree of solubility to the therapeutic agent.

Gelucires are polyglycolyzed glycerides prepared by the alcoholysis reaction of natural oils with polyoxyethylene glycols. They are mixtures of monoesters, diesters and/or triesters of glycerides of long chain C_{12} to C_{18} fatty acids, and in polyethylene glycol mono- and/or diesters of long chain fatty acids. These preparations have a wide range of melting points of from about 33°C to 64°C, as well as a wide range of hydrophilic/lipophilic balance values (HLB) from about 1 to about 14. The Gelucires of particular interest in the present invention have an HLB of above 10.

The first number in the nomenclature of a Gelucire denotes its melting point, whereas the second number provide the HLB value. The preferred Gelucires of the present invention are grades 44/13 and 50/13.

The Pluronic surfactants are block copolymers of polyoxyethylene and polyoxypropylene, generally having an average molecular weight from about 3,000 to about 15,000. The ethoxylated portion of the blocked copolymer generally constitutes from about 30 to about 80% by weight of the molecule. Particularly good results are achievable with Pluronic F68, F108 and F127, but in any case, it is to be noted that the Pluronic constituent should also have an HLB above 10, irrespective of the particular grade which is selected. For example, Pluronic F108 has an average molecular weight of 14,600, a polyoxyethylene content of about 80 weight % and an HLB value in excess of 24, and Pluronic F127 has an average molecular weight of 12,600, a polyoxyethylene content of about 70 weight % and an HLB value from 18 to 23.

The weight ratio of the combined saturated polyglycolyzed glycerides: polyoxypropylene-polyoxyethylene block co-polymer generally range between 0.10-99.9 to 99.9:0.10, with preferred ratios being 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. These combinations especially the 5:5 ratio, yields a mixture having a melting point in the range of 44-70°C, preferably 50°C-70°C.

The composition of saturated polyglycolized glyceride and polyglyoxypropiyene-block copolymer is combined with a therapeutic agent, wherein said composition is present in the final composition, the latter including the therapeutic agent, in a range of about 0.10-99.9% by weight, with the preferred range being 5-75% by weight of the final composition. Poorly soluble agents, e.g., therapeutic agents which have an intrinsic water solubility of less than 10.0 g/l are particularly benefitted by the present invention. Examples of drugs in this category are drugs belonging to the dihydropyridine class of compounds (e.g., nifedepine, felodipine, nicardipine), omperazole, spironolactone, furosemide, terbutaline, riboflavine, gemfibrozil, indomethacin, ibuprofen, phenytoin, glyburide. In addition, any drug which has a water solubility of less than 10.0 g/l belonging to, for example, cardiovascular, cholesterol lowering, anti-hypertensive, antiepileptic, hormonal, hypoglycemic, antiviral, immunosuppressive, antihistaminic, nasal decongestant, antimicrobial, antiarrthrytic, analgesic, antimycobacterial, anticancer, diuretic, antifungal, antiparasitic, protein, peptide, CNS stimulants, CNS depressants, 5-HT inhibitors, anti-schizophrenia, anti-Alzheimer, antipsoriatic, steroidal, oligonucleotide, antiulcer, proton pump inhibitor, anti asthmatic, bronchodialators, thrombolytics, vitamin class of therapeutic agents, any combinations thereof may be used in this composition in order to form solid state solutions and dispersions.

The final composition optionally comprises the following further excipients at 5-95%, especially 10-70% by weight of the final composition. Examples of the further excipients include, but are not limited to ascorbyl palmitate, glycerol, glyceryl monooleate, glyceryl monosterate, glyceryl palmitosterate, triglycerides, diglycerides, monoglycerides, diesters of PEG, monoesters of PEG, polyethylene glycol, glycery polyoxyethlene fatty acid esters, glyceryl polyoxyethylene polyethylene glycol fatty acid esters and ethers, polyoxyethylene alkyl ethers, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene sterates, polyvinyl alcohol, sorbitan fatty acid esters, polyoxyl sterates, polyethylene glycol

hydroxysterate, polyoxyethylene alcohols, anionic, cationic, amphiphillic compounds, carbohydrates (lactose, maltodextrins, sucrose, starch, etc.), polyols (sorbitol, mannitol, xylitol, etc.), microcrystalline cellulose, vitamins (ascorbic acid, niacinamide, etc.), and inorganic compounds (calcium carbonate, dicalcium phosphate), polyoxyethylene castor oil derivatives, propylene carbonate, anionic emulsifying wax, white wax, yellow wax, hydrogenated vegetable oil, triacetin, triethyl citrate and other plasticizers (food and pharmaceutical grade), lecithin, phospholipids, soybean oil, sesame oil, cotton seed oil, sunflower oil, peanut oil, mineral oil, hydrogenated castor oil, water soluble and insoluble derivatives of cellulose (e.g. ethyl cellulose, methyl cellulose, HPMC, HPC, cellulose acetate phthalate, etc.), methacrylates and polymethacrylates (e.g., Eudragit®), canola oil, benzoic acid and its salts, methyl-, propyl- and butyl-para-amino benzoic acid (paraben) (preservatives), organic acids (e.g., fumaric, adipic, maleic, etc.), ethyl alcohol, saccharine, cyclamate sodium, and other artificial sweeteners, food and pharmaceutical flavoring agents, bioflavanoids (e.g., quercetin, isoquercetin), citrus bioflavanoids (e.g., naringin), citrus bioflavanoid complexes, and other agents that inhibit the enzyme cytochrome P450 4A4 (also called as CYP3A4), galactose oligosaccharides (example of a functional carbohydrate), lubricant (e.g. magnesium sterate), anti-caking agent (e.g., silicon dioxide, sodium aluminum silicate, magnesium trisilicate, talc, etc.), gums (locust bean gum, gum arabic, arabinogalactan, etc.); and any combination of said excipients.

To enhance the solubility of the therapeutically active agent, an aspect of this invention provides that the composition of the therapeutic reactive agent, the polyglycolyzed glyceride and the polyoxypropylene-polyoxyethylene block co-polymer is formed into a solid state solution or solid state dispersion.

A solid state solution is defined as a solution of the drug in a solid form. A solid state solution of a drug is characterized by the lack of a melting point peak at the melting point of the drug indicating the absence of the solid state of the drug. A solid state solution-dispersion is defined as a system in which part of the system may be in the solid solution form and part of it may be in the form of a finely dispersed solid form in the system. This solid state solution - dispersion is further defined as a system in which more than 1% of the total drug content can exist as a solid solution and more than 1% of the drug can exist as a solid dispersion with a particle size distribution such that 90% of the

particles have a diameter less than 10 microns. The weight ratio between the solid state solubilized drug: dispersed drug may be in the range of 1.0-100:99-0. It is desirable that 30-100% of the drug exists as a solid state solution. The ratio of the amount of drug present in the form of a solid state solution to the amount present as a solid dispersion is easily ascertained by the use of techniques in thermal analysis such as Differential Scanning Calorimetry (DSC), Thermal Gravimetric Analysis (TGA), and Differential Scanning Microcalorimetry. The crystallinity of the drug is easily determined by X-ray diffraction. Furthermore, when determined by thermal analytical techniques it is desirable that the final composition have at least "one" distinct melting peak in the range of 30-80°C associated with the melting point of the non-drug components of the final composition.

To produce the final composition polyglycolyzed glycerides and polyoxypropylene-polyoxyethylene block co-polymer are heated sufficiently to form a melt of the ingredients, for example to at least about 20°C above the combined melting point. The therapeutic agent is added gradually to the molten mixture of polyglycolyzed glycerides and polyoxypropylene-polyoxyethylene block co-polymer. It is preferable to mill or micronize the drug to a particle size range such that the particle diameter of 90% of the particles is less than 75 microns. The mixture is maintained at a sufficient temperature, for example, at least about 20°C above the combined melting point of the polyglycolyzed glycerides: polyoxypropylene-polyoxyethylene block co-polymer mixture for a sufficient time to dissolve or disperse the pharmaceutical agent.

The optional excipients may be added to the above mentioned system to,

(A) Increase the solubility of the drug in the polyglycolyzed glycerides: polyoxypropylene-polyoxyethylene block co-polymer system.

(B) Set the melting point of the non-drug components, such that at least "one" melting point peak belonging to the non-drug components is present between 30-80°C in the final composition, when analyzed by thermal analytical techniques.

The resulting mixture is then maintained in the molten form, for example, at least 20°C above the combined melting point of the non-drug components, with constant

stirring to ensure homogenous distribution of the drug in the system. Thereafter, the mixture may be subjected to one or more of the following operations:

I) Allowed to congeal to a solid mass, and then extruded through a hot melt extruder into a powder.

II) Milled using equipment that would maintain the milling conditions at room temperature or below the melting point of the non drug components of the system to enable milling the composition into a powder.

III) Spray congealed in a spray drier or fluidized bed drier to a powder.

IV) Congealed onto one or more of said excipients in a spray drier, fluidized bed drier, rotor, high shear granulator, planetary mixer, blender, or any conventional food and pharmaceutical processing equipment.

V) The above mentioned powders may then be used in the formulation of conventional, specialized, and novel pharmaceutical dosage forms such as tablets, capsules, powders for inhalation, suppositories, suspensions, and emulsions.

VI) Alternatively, the molten mixture can be congealed into or onto a solid pharmaceutical dosage form such as, for example, a tablet, capsule, and/or granule.

The entire disclosure of all applications, patents and publications, cited above and below, and also U.S. provisional application 60/063,338 filed October 27, 1997 are hereby incorporated by reference.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

In the foregoing and in the following examples, all temperatures are set forth uncorrected in degrees Celsius and unless otherwise indicated, all parts and percentages are by weight.

EXAMPLES

Example 1:

Three grams of Gelucire of 50/13 and 3 grams of Pluronic F68 are melted and two grams of nifedipine are dissolved therein. The resultant solution is added to 4 grams of sorbitol while stirring. The resultant solution is then cooled down and passed through a 20 mesh screen. The resultant particulate solids were solid solutions.

Example 2:

In this example, felodipine is employed as the active pharmaceutical agent. Thus, 1.5 grams of Gelucire 50/13 and 1.5 grams of Pluronic F68 were melted together and 1 gram of felodipine was added thereto. This resultant solution was then introduced into 4 grams of Sorbitol P300® while stirring. After mixing, the solution was cooled and ultimately passed through a 20 mesh screen. The resultant particulate solids were in a solid state solution-dispersion system.

Example 3:

In this example, felodipine is employed as the active pharmaceutical agent. Thus, 1.5 grams of Gelucire 50/13 and 1.5 grams of Pluronic F68 were melted together and 1.5 grams of felodipine was added thereto. This resultant solution was then introduced into 4 grams of Sorbitol P300® while stirring. After mixing, the solution was cooled and ultimately passed through a 20 mesh screen. The resultant particulate solids were in a solid state solution system.

Example 4:

In this example, felodipine is employed as the active pharmaceutical agent. Thus, 1 gram of Gelucire 50/13 and 1 gram of Pluronic F68 were melted together and 1.5 grams of felodipine was admixed. This resultant solution was then added to 2.67 grams of Avecil, a brand of microcrystalline cellulose. After mixing, the solution was cooled and ultimately passed through a 20 mesh screen. The resultant particulate solids were in a solid state solution-dispersion system.

For the purposes of increasing the melting point of the composition of Examples 2, 3 and 4, Pluronic F127 can be substituted for Pluronic F68.

To determine the improvement achieved by the present invention with respect to the solubility of pure felodipine, the formulations of Examples 3 and 4 were evaluated by the following technique.

The solubility characteristics of the compositions of Examples 3 and 4 as compared to pure felodipine were evaluated, as follows:

- 6.8 mg of felodipine and 34.8 mg of the composition of Example 3 and 34.2 Example 4 are respectively placed into 500 ml of a 40% PEG solution maintained at 37°C.

The solution was stirred with a paddle stirrer at 50 rpm. The absorbency was measured with a Hitachi spectrometer at 362 nm. The percent release is based on the standard curve: absorbents = 0.216 conc. (mg/900 ml)-0.00274, and the following results were obtained.

Percent released (%)			
Time (h)	Pure felodipine	Example 3	Example 4
0.5	9.2	62.4	65.6
1	21.1	76.4	78
2	49.5	84.4	84.6
3	66.8	85.7	86.3

From the above table, it is clear that the present invention provides a substantially enhanced solubility as compared to the pure drug.

Example 5:

For the production of tablets 121.5 mg of HPMC (hydropropylmethyl cellulose) are mixed with 21.1 gm of Sorbitol Instant P300®, 246.88 mg of Microcrystalline Cellulose and 59.62 mg of the composition of Example 3. 1 mg of magnesium stearate was then added to the above mixture with stirring. The resultant mixture was tableted in a Carver® press under a pressure of 2 tons.

Example 6:

Another tablet was produced from the composition of Example 3 by the method of Example 5 except in this case, the amount of Example 3 was 59.52 mg, Microcrystalline Cellulose 231.98 mg, Sorbitol Instant P300® 45 mg and HPMC 112.5 mg, with sodium stearyl fumerate (PRUV)® being substituted for magnesium stearate.

The resultant tablet has 10% sorbitol.

Example 7:

In this example, a 15% sorbitol tablet is produced in the same manner as the last example except that the amount of sorbitol is 67.5 mg and the amount of Microcrystalline Cellulose is 209.48 mg.

Example 8: Intrinsic Dissolution of Hydrosolve-Ibuprofen

a. Formulation:

Excipient	Quantities (g)	Supplier
Ibuprofen	2.5	Albermarle
Pluronic F68	2	BASF
Gelucire 50/13	0.5	Gatterfosse
Sorbitol Instant P300	5	EM Industries

b. Procedures:

1.Melt Gelucire and Pluronic, and dissolve ibuprofen into the mixture.

2.Add the above solution into Sorbitol Instant while stirring.

3.Cool down and pass through #20 mesh screen.

c. Dissolution Test Using the Dissolution Test as follows:

1.Measure 3.3 mg and 17.2 mg of ibuprofen (20 micron) and HydroSolve - Ibuprofen, respectively.

2.Measure the dissolution profiles using 700 ml of pH 7.2 buffer and paddle at 50 rpm.

3.Measure the absorbance at 221 nm.

Results:

Time(min)	Ibuprofen	HydroSolve
10	75.93	85.19
20	81.14	95.28
30	81.14	99.07

Example 9: Intrinsic Dissolution Profile of Phenytoin and HydroSolve- Phenytoin

a. Formulation #4:

Excipient	Quantities (g)	Supplier
Phenytoin	1	Spectrum Quality Products
Gelucire 50/13	1	Gattefosse
Pluronic F68	1	BASF

b. Procedures:

1. Melt Gelucire and Pluronic together.
2. Dissolve the phenytoin into above solution.
3. Congeal this suspension and pass through #20 mesh.
4. Measure about 32 and 110 mg of phenytoin and HydroSolve - phenytoin, respectively.
5. Measure the dissolution profiles using 900 ml of D1 water and Paddle Method at 50 rpm.
6. Using D1 water as blank, measure the absorbance at 220 nm of each sample which is filtered through 0.45 micron filters.
7. Calculate the percent released by standard curve:

   Absorbance = 0.4072 x Concentration (mg/100ml) + 0.0227

c. Results:

	% Dissolved (%)	
Time (h)	Phenytoin	HydroSolve
0.5	3.03	60.67
1	5.92	67.27
2	14.42	72
3	20.58	73.38

Example 10: The DSC Profiles for HydroSolve System of Phenytoin

1a. Formulation #1:

Excipient	Quantities (g)	Supplier
Phenytoin	1	Spectrum Quality Products
Gelucire 50/13	1	Gatterfosse
Pluronic F68	1	BASF
Sorbitol Instant P300	2.5	EM Industries

2b. Procedures:

1.Melt Gelucire and Pluronic together.

2.Disperse the phenytoin into above solution since pheytoin cannot completely.

3.Mix the above suspension with sorbitol P300 while stirring.

4. Measure the DSC profile.

2a. Formulation #4:

Excipient	Quantities (g)	Supplier
Phenytoin	1	Spectrum Quality Products
Gelucire 50/13	1	Gatterfosse
Pluronic F68	1	BASF

2b. Procedures:

1.Melt Gelucire and Pluronic together.

2.Dissolve/disperse the phenytoin into above system.

3.Congeal this suspension and pass through #20 mesh.

4. Measure the DSC profile.

c. Results:

	PEAKS	
Material	Endotherm (°C)	Exotherm (°C)
Phenytoin:	298.5	
Pluronic F68:	56.6	163.4
F#1:	52.3	179.2
F#4:	52.4	174.2

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

CLAIMS

What is claimed is:

1. A pharmaceutical excipient suitable as a solubility enhancer for a pharmaceutical composition comprising a poorly water soluble therapeutically active compound, said excipient comprising a mixture of:

(a) polyglycolyzed glycerides, and

(b) polyoxypropylene-polyoxyethylene block copolymers.

2. An excipient of claim 1, wherein (a) is a Gelucire composition.

3. An excipient of claim 1, wherein (b) is a Pluronic surfactant.

4. An excipient of claim 1, wherein (a) is a Gelucire composition wherein the composition of the C_{8-18}-long-chain fatty acids in the glycerides comprises <10% C_8-fatty acid, <10% C_{10}-fatty acid, <50% C_{12}-fatty acid, <25% C_{14}-fatty acid, <50% C_{16}-fatty acid, and <58% C_{18}-fatty acid.

5. An excipient of claim 1, wherein (a) is a Gelucire composition wherein the composition of the C_{8-18}-long-chain fatty acids in the glycerides comprises at least one of polyethylene glycol mono- or diesters of said fatty acids.

6. An excipient of claim 4, wherein (a) is a Gelucire composition wherein the composition of the C_{8-18}-long-chain fatty acids in the glycerides comprises at least one of polyethylene glycol mono- and diesters of said fatty acids.

7. A pharmaceutical composition comprising a solid state solution or solid state dispersion of a poorly water soluble therapeutically active compound and a pharmaceutically acceptable excipient, said excipient comprising a mixture of:

(a) polyglycolyzed glycerides, and

(b) polyoxypropylene-polyoxyethylene block copolymers,

whereby the solubility of the poorly soluble therapeutically active compound in the pharmaceutical composition is enhanced.

8. A pharmaceutical composition of claim 7, wherein (a) is a Gelucire composition.

9. A pharmaceutical composition of claim 7, wherein (b) is a Pluronic surfactant.

10. A pharmaceutical composition of claim 7, wherein (a) is a Gelucire composition wherein the composition of the C_{8-18}-long-chain fatty acids in the glycerides comprises <10% C_8-fatty acid, <10% C_{10}-fatty acid, <50% C_{12}-fatty acid, <25% C_{14}-fatty acid, <50% C_{16}-fatty acid, and <58% C_{18}-fatty acid.

11. A pharmaceutical composition of claim 7, wherein (a) is a Gelucire composition wherein the composition of the C_{8-18}-long-chain fatty acids in the glycerides comprises at least one of polyethylene glycol mono- and diesters of said fatty acids.

12. A pharmaceutical composition of claim 10, wherein (a) is a Gelucire composition wherein the composition of the C_{8-18}-long-chain fatty acids in the glycerides comprises at least one of polyethylene glycol mono- and diesters of said fatty acids.

13. A pharmaceutical composition of claim 7, wherein the composition comprising the solid state solution or solid state dispersion of a poorly water soluble therapeutically active compound and the excipient is granulated, pelleted, extruded, extrusion spheronized, or spray congealed.

14. A pharmaceutical composition of claim 7, wherein the composition comprising the solid state solution or solid state dispersion of a poorly water soluble

therapeutically active compound and the excipient is combined with an agent that modifies the release profile of the therapeutically active compound.

15. A pharmaceutical composition of claim 14, wherein the agent that modifies the release profile of the therapeutically active compound is a polymer of cellulose or a derivative thereof, alginic acid or a derivative thereof, polyvinyl alcohol or a derivative thereof, acrylic acid polymer, polymethacrylates, acrylic acid or a derivative thereof, lactic acid or a derivative thereof or gelatin.

16. A pharmaceutical composition of claim 14, wherein the composition comprising the solid state solution or solid state dispersion of a poorly water soluble therapeutically active compound and the excipient is in the form of a granule, particle, pellet, tablet or sphere, and is coated with the agent that modifies the release profile of the therapeutically active compound.

17. A method for manufacturing an excipient for a pharmaceutical composition, said composition comprising a solid state solution or solid state dispersion of a poorly water soluble therapeutically active compound, said excipient comprising a mixture of:

(a) polyglycolyzed glycerides, and

(b) polyoxypropylene-polyoxyethylene block copolymers,

whereby the solubility of the poorly soluble therapeutically active compound in the pharmaceutical composition is enhanced, comprising:

heating said polyglycolyzed glycerides and polyoxypropylene-polyoxyethylene block co-polymer sufficiently to melt the ingredients,

adding the therapeutic agent to the molten mixture of polyglycolyzed glycerides and polyoxypropylene-polyoxyethylene block co-polymer

maintaining the mixture at a sufficient temperature for a sufficient time to dissolve or disperse the pharmaceutical agent.

18. A method of claim 17, further comprising adding one or more optional excipient, whereby:

(A) the solubility of the therapeutically-active compound in the polyglycolyzed glyceride:polyoxypropylene-polyoxyethylene block co-polymer mixture is increased, or

(B) the melting point of the non-drug components is set, whereby at least one melting point peak belonging to the non-drug components is present between 30-80°C in the final composition, when analyzed by thermal analytical techniques.

19. A method of claim 17, further comprising

maintaining the resulting mixture in the molten form, with constant stirring to ensure homogenous distribution of the drug in the system, and then

subjecting the molten mixture to one or more of the following operations:

I) allowing the mixture to congeal to a solid mass, and then extruding the mixture through a hot melt extruder into a powder;

II) milling the mixture using equipment that would maintain the milling conditions at room temperature or below the melting point of the non-drug components of the system to enable milling the composition into a powder;

III) spray-congealing the mixture in a spray drier or fluidized bed drier to a powder;

IV) congealing the mixture onto one or more optional excipients in a spray drier, fluidized bed drier, rotor, high shear granulator, planetary mixer, blender, or any conventional food and pharmaceutical processing equipment;

V) formulating the above-mentioned powders in pharmaceutical tablets, capsules, powders for inhalation, suppositories, suspensions, and emulsions; or

VI) congealing said mixture onto a solid pharmaceutical tablet, capsule or granule.

20. A pharmaceutical composition according to claim 7 in the form of a solid state solution.

21. A pharmaceutical composition according to claim 7 in the form of a solid state dispersion.

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/EP 98/06544

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MAES, P. ET AL: "In vitro and in vivo behavior of some liquid or semi-solid filled hard gelatin capsules" BULL. TECH./GATTEFOSSE REP. (1996), 89, 63-70 CODEN: BTGRDQ;ISSN: 0397-7617, XP002099025 see page 67 ---	1-13,17, 20,21
A	DORDUNOO, S. K. ET AL: "Preformulation studies on solid dispersions containin triamterene or temazepam in polyethylene glycols or gelucire 44/14 for liquid filling of hard gelatin capsules" DRUG DEV. IND. PHARM. (1991), 17(12), 1685-713 CODEN: DDIPD8;ISSN: 0363-9045, XP002099026 see page 1689 - page 1690 ---	1-21
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 April 1999	16/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Boulois, D

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INTERNATIONAL SEARCH REPORT

Inte cnal Application No
PCT/EP 98/06544

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 43635 A (GAUTIER JEAN CLAUDE ;SANOFI SA (FR); MARRIER JEAN MARIE (FR)) 8 October 1998 see page 3, line 9 - page 4, line 5 ---	1-13,17, 20,21
X	WO 96 21439 A (GALEPHAR P R INC ;DEBOECK ARTHUR M (PR); BAUDIER PHILIPPE (BE); MA) 18 July 1996 see page 9; example 1 ---	1-12,17, 19-21
X	US 5 487 887 A (BENFATTO ANTHONY) 30 January 1996 see column 9; example 5 ---	1-6
A	GINES, J. M. ET AL: "Elaboration and thermal study of interactions between cinnarizine and Gelucire 53/10 physical mixtures and solid dispersions" INT. J. PHARM. (1995), 126(1,2), 287-91 CODEN: IJPHDE;ISSN: 0378-5173, XP002099028 see the whole document -----	1-21

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ional Application No
PCT/EP 98/06544

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 7052698 A	22-10-1998
WO 9621439 A	18-07-1996	US 5545628 A	13-08-1996
		AU 4380896 A	31-07-1996
		CA 2210985 A	18-07-1996
		EP 0801562 A	22-10-1997
		JP 10511959 T	17-11-1998
US 5487887 A	30-01-1996	US 5575990 A	19-11-1996

PHARMACEUTICAL TECHNOLOGY, May 1984

Considerations in selecting antimicrobial preservative agents for parenteral product development

MICHAEL J. AKERS

AMONG SEVERAL important characteristics of a parenteral product, sterility is the most essential. For parenteral products terminally sterilized and intended for single dose injection, maintenance of sterility is a function of both the method of sterilization and the integrity of the package system. For parenteral products that cannot be terminally sterilized and/or that are intended for multiple dosing, however, antimicrobial agents must be added to the product formulation to protect the product from accidental microbial contamination during its manufacture, shelf life, and use.

The selection of the best antimicrobial

Table I: Properties of the ideal preservative.

- Effective in low concentrations against a wide variety of microorganisms
- Soluble in the formulation at the required concentration
- Nontoxic and nonsensitizing externally and internally in the concentrations required
- Compatible with a wide variety of drugs and solubilizing and dispersing agents
- Free from objectionable odor, taste, or color
- Active with long-term stability over a wide range of pH and temperature
- Inexpensive
- Nonreactive with components of the container/closure system

Table II. Antimicrobial agents for parenterals.

Antimicrobial Agent	Recommended Concentration in Some Pharmacopeias			
	USP*		BP† (%)	FP‡ (%)
	(%)	Ophthalmic Solution		
Phenol	+§	−‖	0.5	0.5
Cresol	−	−	0 3	0.3
p-Chloro-*m*-cresol	−	−	0.1	0 3
Phenylethyl alcohol	+	0.5	−	−
Chlorobutanol	−	0 5	−	−
Benzyl alcohol	−	−	−	1.0
Methylparaben	+	−	−	0.15
Propylparaben	+	−	−	0.15
Phenylmercuric acetate, borate, nitrate	+	0.002	0 001	+
Thimerosal	+	−	−	−
Benzalkonium chloride	+	0.01	−	−

* *United States Pharmacopeia XX*, 1980
† *British Pharmacopoeia*, 1980
‡ *French Pharmacopoeia IX*, 1976
§ + = listed as an antimicrobial agent, no limit specified
‖ − = not listed as a recommended antimicrobial agent, although this does not necessarily mean the agent is unacceptable

agent for the product being developed presents a difficult task for the development scientist. There certainly exists no ideal antimicrobial preservative; that is, a preservative meeting all of the criteria listed in Table I.[1] Because of toxicity and solubility issues, relatively few antimicrobial agents are acceptable for parenteral administration. Indeed, no new preservative for parenteral formulation has been introduced during the past 25 years.

Regulatory agencies in the United States and abroad are enforcing stricter regulations regarding the testing and stability requirements of preserved parenteral drug products. The British Pharmacopoeia (BP) preservative efficacy test (PET) requires a 10^1 reduction in the surviving bacterial population within 6 hr following inoculation of the product with at least 10^6 bacterial cells.[2] In contrast, the United States Pharmacopeia (USP) requires the same 10^1 reduction in bacterial cells within 14 days.[3] In the United States, however, FDA is becoming more demanding in requiring the pharmaceutical industry to validate that the parenteral product containing a preservative system will pass the PET at the end of its shelf life.

Preservation of pharmaceutical products has been the subject of several good review articles, as well as an international symposium.[4-6] To date, however, no thorough review article on the use of preservatives exclusively in the parenteral product areas has been published. To fill that gap, this paper will provide an update of the current uses, problems, and evaluation of preservatives in parenteral products.

Current Usage of Parenteral Antimicrobial Preservatives

There are five main antimicrobial agent classes applicable to parenteral product formulation:

- alcohols and their substituted and halogenated derivatives
- benzoic acid derivatives and their esters
- phenolic compounds
- quaternary ammonium compounds
- mercury organic compounds.

Recommended concentrations of specific antimicrobial agents published by various compendia are given in Table II.

The antimicrobial preservative agents used in today's commercially available parenteral products are listed in Table III. The most widely used antimicrobial agent is

Table III: Specific uses and concentrations of antimicrobial agents in some commercially available parenteral products (from Physicians' Desk Reference, *1983).*

Antimicrobial Preservative	Brand Name Product	Concentration or Amount of Preservative	Manufacturer
Phenol	Acthar	0.5%	Armour
	APL	0.2%	Ayerst
	Calphosan	0.25%	Carlton
	Nebcin	0.5%	Dista
	Drolban	0.5%	Lilly
	Ergotrate	0.25%	Lilly
	Glucagon, diluent	0.2%	Lilly
	Humulin N	0.065%	Lilly
	NPH Iletin I	0.065%	Lilly
	Protamine, Zinc, and Iletin I	0.25%	Lilly
	Quinidine Gluconate	0.25%	Lilly
	Imferon	0.5%	Merrell Dow
	Konakion	0.45%	Roche
	Nisentil	0.45%	Roche
	Prostigmin	0.45%	Roche
	Synkayvite	0.45%	Roche
	Tensilon	0.45%	Roche
	Tagamet	0.5%	SKF
	Mepergan	5 mg	Wyeth
	Phenergan	5 mg	Wyeth
m-Cresol	Humulin N	0.16%	Lilly
	Humulin R	0.25%	Lilly
	Iletin II U500	0.25%	Lilly
	NPH Iletin I	0.16%	Lilly
	Regular Iletin	0.25%	Lilly
	Demerol	0.1%	Winthrop
Benzyl alcohol	A-Hydrocort	0.9%	Abbott
	A-Methapred	0.9%	Abbott
	Erythrocin Lactobionate	180 mg/1 g erythromycin	Abbott
	APL	2.0%	Ayerst
	Septra	1.0%	Burroughs Wellcome
	Sotradecol	2.0%	Elkins-Sinn
	Amicar	0.9%	Lederle
	Aristocort	0.9%	Lederle
	Aristospan	0.9%	Lederle
	Leucovorin	0.9%	Lederle
	Methotrexate	0.9%	Lederle
	Duracillin	1.0%	Lilly
	Heparin	1.0%	Lilly
	Oncovin IV	0.9%	Lilly
	Velban	0.9%	Lilly
	Aquamephyton	0.9%	Merck
	Hydeltra T.B.A.	0.9%	Merck
	Hydrocortone ophthalmic ointment	0.5%	Merck
	Myochrysine	0.5%	Merck
	Deca-durabolin (50 mg)	10.0%	Organon
	Durabolin (25 mg)	5.0%	Organon

continued

Table III (continued).

Antimicrobial Preservative	Brand Name Product	Concentration or Amount of Preservative	Manufacturer
Benzyl alcohol	Regonol	1.0%	Organon
	Vistaril	0.9%	Pfipharmecs
	Robinul	0.9%	Robins
	Bactrim	1.0%	Roche
	Berocca	1.0%	Roche
	Librium	1.5%	Roche
	Valium	1.5%	Roche
	Geopen	0.9%	Roerig
	Navane	0.9%	Roerig
	Ditate	2.0%	Savage
	Compazine	0.75%	SKF
	Kenalog 40	0.9%	Squibb
	Pronestyl	0.9%	Squibb
	Velosef	0.9%	Squibb
	Cleocin Phosphate	0.945%	Upjohn
	Solu-medrol	0.88%	Upjohn
	Heparin	1.0%	Wyeth
Chlorobutanol	Epitrate	0.55%	Ayerst
	Phospholine Iodine	0.5%	Ayerst
	Dolophine	0.5%	Lilly
	Hexa-Betalin	0.5%	Lilly
	Tubocurarine chloride	0.5%	Lilly
	Bentyl	0.5%	Merrell Dow
	Adrenalin	0.5%	Parke-Davis
	Dopram	0.5%	Robins
	Syntocinon	0.5%	Sandoz
Benzalkonium chloride	Clear eyes	0.01%	Abbott
	Murine	0.01%	Abbott
	Decadron	0.02%*	Merck
	Hydeltrasol	0.02%	Merck
	Hydrocortone	0.02%	Merck
	Neo-Hydeltrasol	0.02%	Merck
	Timoptic	0.01%	Merck
	Metimyd	0.025%	Schering
	Most products	0.01%	Alcon and Allergan
Thimerosal	Cortisporin	0.001%	Burroughs Wellcome
	Neosporin	0.001%	Burroughs Wellcome
	Percorten	0.002%	CIBA
	Pnu-Imune	0.01%	Lederle
	Diuril	0.4 mg	Merck
	Edecrin	0.1 mg	Merck
	MICRhoGAM	0.01%	Ortho
	RhoGAM	0.01%	Ortho
	Collyrium	0.002%	Wyeth
	Wydase	0.075%	Wyeth
Phenylmercuric nitrate acetate	Estradurin	0.022 mg	Ayerst

*Also contains phenylethanol 0.25%

continued

Table III (continued).

Antimicrobial Preservative	Brand Name Product	Concentration or Amount of Methylparaben	Concentration or Amount of Propylparaben	Manufacturer
Parabens	Marcaine	0.1%	—	Breon
	Apresoline	0.065%	0.035%	CIBA
	Nubain	0.18%	0.02%	Endo
	Numorphane	—	0.02%	Endo
	Inapsine	0.18%	0.02%	Janssen
	Monistat	0.05%	0.005%	Janssen
	Duracillin	0.15%	0.02%	Lilly
	Lente Iletin	0.1%	—	Lilly
	Decadron	0.15%	0.02%	Merck
	Elavil	0.15%	0.02%	Merck
	Permaden	0.09%	0.01%	Pfipharmecs
	Pfizerpen AS	0.103%	0.011%	Pfipharmecs
	Levo Dromoran	0.18%	0.02%	Roche
	Mestinon	0.18%	0.02%	Roche
	Prostigmin	0.18%	0.02%	Roche
	Garamycin	0.18%	0.02%	Schering
	Solganal	—	0.1%	Schering
	Crysticillin	0.13%	0.02%	Squibb
	Prolixin	0.1%	0.01%	Squibb
	Velosef	0.12%	0.014%	Squibb
	Solu-B	0.13%	0.015%	Upjohn
	Talwin	0.1%	—	Winthrop
	Bicillin CR	0.12%	0.014%	Wyeth
	Bicillin LA	0.12%	0.014%	Wyeth
	Wycillin	0.14%	0.015%	Wyeth

benzyl alcohol, with the parabens a distant second. The advantages and disadvantages of various antimicrobial agents have been discussed elsewhere.[1] A summary of the activity and optimal pH range of these agents is given in Table IV.[7] In addition, biological parenteral products such as toxoids, blood products, and vaccines contain the following antimicrobial preservatives with common percentages: benzyl alcohol (0.9%), methylparaben (0.1%), thimerosal (0.005%), and 2-phenoxy-ethanol (0.375%).[4]

Table IV: Activity of parenteral preservatives as a function of type of microorganism and product pH. Key: 1 = good effectiveness, 2 = moderate effectiveness, 3 = ineffective; GPB = gram-positive bacteria, GNB = gram-negative bacteria (from Wallhausser, K.-H., Develop. Biol. Standard., Vol. 24, Basel, S. Karger, 1974, pp. 9–28).

Agent	Concentration (%)	Microorganism				pH		
		GPB	GNB	Yeasts	Fungi	2–4	5–7	8–10
Phenol	0.3	1	1	2	2	1	1	1
Cresol	0.3	1	1	1	1	1	1	1
Chlorobutanol	0.5	1	1	2	2	1	3	3
Benzyl alcohol	1.0	1	2	2	2	1	2	3
Methylparaben	0.18	1	1	3	3	1	1	2
Propylparaben	0.02	1	1	2	3	1	1	2
Methyl + *p*-paraben	0.2	1	1	2	2	1	1	2
Methyl + *p*-paraben and benzyl alcohol	0.2+ 0.5	1	1	1	1	1	1	3
Phenylmercuric nitrate	0.001	1	1	1	1	3	3	1
Thimerosal	0.02	1	1	1	1	1	1	2
Benzalkonium chloride	0.01	1	2	1	1	2	1	2

Phenolics (Phenol, Cresol)
• Excessive light exposure will catalyze the oxidation of the phenolic hydroxy group • Will volatilize through a rubber closure having a high vapor transmission coefficient • Potentially physically incompatible with certain nonionic surfactants
Benzyl Alcohol
• Sensitive to excessive light exposure • Will volatilize through rubber closures having high vapor transmission coefficients • Steam sterilization can catalyze oxidation
Chlorobutanol
• Sensitive to excessive light exposure • Easily volatile • Unstable at solution pH levels greater than 6 • Diffuses through, or is sorbed by, polyethylene and polypropylene
Parabens
• Sensitive to excessive light exposure • Chemical stability decreases as pH increases • Will bind to various macromolecular polymers • Incompatible with alkaline excipients and polysorbate 80 • Tend to migrate into certain types of rubber closures
Quaternary Ammonium Compounds
• Not used in injectable dosage forms; used only in topical ophthalmic dosage forms • Incompatible with anionic and nonionic surfactants, tartrates, nitrates, alkalis, and certain rubber components • Tendency to adsorb to membrane filters
Thimerosal
• Sensitive to excessive light and air exposure • Can be oxidized by various trace metal ions, especially copper, and by alkaline pH • Incompatible with acids, heavy metal salts, many alkaloids • Can adsorb to various types of rubber closures
Phenylmercuric Salts
• Sensitive to excessive light and air exposure • Incompatible with halide salts • Can adsorb to polyethylene and certain rubber components

Table V: The stability/compatibility limitations of preservative agents.

Agent	Amount (mg/ml)
Phenol	5.0
Metacresol	2.5
Benzyl alcohol	1000 0
Chlorobutanol	5.5
Methylparaben	2.56
Propylparaben	0 35
Butylparaben	0.15
Thimerosal	0.4
Phenylmercuric nitrate	0 022
Benzalkonium chloride	0.025
Benzethonium chloride	0 1

Table VI: Maximum acceptable concentrations for antimicrobial agents.

Problems with Antimicrobial Preservatives

Stability and compatibility. The inherent reactive chemical nature of preservative compounds results in major stability/compatibility problems, thus limiting any single preservative agent from becoming the ideal. On the one hand, stability problems primarily are solvent-related, involving volatilization or either hydrolytic or oxidative degradation. Manufacturing and sterilization processes often are potential sources of antimicrobial agent degradation. On the other hand, compatibility problems primarily involve interactions with packaging components or other solutes in the formulation when those other solutes have relatively high surface activity

In deciding upon an appropriate preservative agent, the formulator should appreciate the various stability/compatibility limitations of each potential agent, which are summarized in Table V

Toxicity The fact that antimicrobial agents have the ability to destroy microbial cells obviously classifies these agents as potentially harmful to man. *All* antimicrobial agents currently available for parenteral application possess potentially serious toxicity and/or stability limitations, which is why at least three major restrictions regarding the use of preservatives are enforced by regulatory agencies. Those restrictions stipulate:

- recommended or maximum concentration allowed in the parenteral formulation
- maximum volume of solution allowed for injection (no more than 15 ml)
- prohibited routes of administration for parenterals containing antimicrobial agents — including intraocular, intracardiac, and intrathecal routes.

Fortunately, the amount of preservative required to destroy microbial life falls within a range that is relatively nontoxic to humans. While it is not the case in the United States, some countries specify the maximum acceptable concentration for various antimicrobial agents in parenteral products (Table II). These figures are good indicators of the level of preservative concentration above which toxic reactions could begin in man. The maximum known concentrations of various parenteral preservatives marketed in the United States are listed in Table VI: despite the use of preservatives in con-

centrations at or below the levels stated in these tables, toxicity attributable to misuse of the product and sensitization reactions occurring in certain patients continue to be reported.

For *phenol and other phenolic compounds*, it is generally believed that solutions above 1.0% are bactericidal depending upon the type of organism and the temperature. While phenol concentrations at 0.5% and lower are considered to be bacteriostatic and relatively nontoxic, there are nonetheless some reports of phenol toxicity even at these low concentrations.

Phenol in glucagon diluting solution exists at a maximum concentration of 0.2% — a harmless amount when glucagon is administered for treatment of hypoglycemia or is used in diagnostic gastrointestinal examinations. But larger doses of glucagon, such as those required in treatment of myocardial contractile failure, produced toxic responses in patients because of the correspondingly larger doses of phenol administered with the reconstituted glucagon.[10,11]

Allergenic extracts preserved with phenol have been reported to cause sensitization responses in patients given repeated injections of these extracts.[12] Such responses include extreme dizziness, ataxia, severe headache, depression, and, ultimately, modifications in the immune response (immunosuppression). These reports, however, did not identify the allergenic products, the labeled strength of phenol in these products, nor the number and amount of doses administered.

Chlorocresol was once a common preservative agent in ophthalmic dosage forms. It has, however, been deleted from *BP*, and it also has been reported to cause hypersensitivity reactions following intravenous injection.[13]

Benzyl alcohol is regarded as one of the least toxic of all available parenteral antimicrobial agents. Indeed, a single dose of 30 ml of 0.9% benzyl alcohol is considered safe for healthy adult humans,[14] and benzyl alcohol is used as the bacteriostatic agent in bacteriostatic diluents such as water for injection and 0.9% sodium chloride.

Before 1981, benzyl alcohol toxicity was recognized only when using much larger doses than the range of 0.5 ml/kg produced by 30 ml of 0.9% benzyl alcohol solution. In 1981, however, Gershanik et al. reported that benzyl alcohol caused a fatal toxic syndrome (*gasping syndrome*) in premature infants.[15] Such infants had been administered several injections of bacteriostatic sodium chloride for flushing intravascular catheters as well as medications reconstituted with diluents containing 0.9% benzyl alcohol. The average daily quantities of benzyl alcohol received in gasping infants was between 99 mg/kg and 234 mg/kg, a daily dose of benzyl alcohol that was from 20 times to 50 times the 0.5 ml/kg dose mentioned above as safe in adult humans.

In a follow-up study, these same investigators found that the onset and extent of gasping syndrome were directly a result of benzyl alcohol accumulated in the blood through large doses of benzyl alcohol (99–234 mg/kg) relative to patient body size and the reduced ability of infants to metabolize and detoxify benzyl alcohol.[16] Their conclusion, obviously, was to eliminate the use of bacteriostatic diluents as flushing or reconstitution agents in neonatal therapy, a determination supported by FDA's request that all products containing benzyl alcohol essentially be removed from neonatal treatment areas.

Benzyl alcohol toxicity in immature animals recently was reported when these animals were intravenously treated for dehydration with lactated Ringer's solution containing 1.5% benzyl alcohol.[17] Furthermore, as little as 0.25 ml of lactated Ringer's solution containing 1.5% benzyl alcohol injected intraperitoneally was lethal to weanling mice, although mature mice were not affected when injected with similar doses of lactated Ringer's solution containing benzyl alcohol.

Like benzyl alcohol, the *parabens* exhibit a very low order of toxicity and are considered safe at the concentrations used in preserving parenteral products. The most noted side effect of parenterally administered parabens, however, has been their capability of producing allergic reactions.[18,19] Toxicity of parabens increases as the length of the alkyl group increases; the butyl ester is about three times as toxic as the methyl ester.

Chlorobutanol has been reported to cause hypersensitivity in patients given heparin injection.[20] At 0.5% concentrations, chlorobutanol in sodium chloride injection will cause total hemolysis after a 45-min exposure.[21] Chlorobutanol also has been reported to cause eye irritation upon ophthalmic topical administration.[22]

Thimerosal has been reported to cause delayed hypersensitivity reactions in nine of 44 patients injected intradermally with 0.01% thimerosal solutions.[23] The inclusion of mercury in thimerosal as well as in the phenylmercuric salts has led to minimal usage of those preservatives in parenteral modifications because of fears of potential mercury poisoning. In fact, FDA intends to

Table VII: Comparison of 1980 BP and 1980 USP tests for efficacy of preservatives in formulated products

Factor	1980 BP	1980 USP
Products affected	1. Single- or multiple-dose parenteral 2 Ophthalmic 3. Topical 4. Liquid oral	1. Multiple-dose 2. Ophthalmic 3. Otic 4 Nasal
Test application	The complete product over claimed shelf life and the likely period of use; repeat challenges may be appropriate	Original unopened container in which it was distributed by the producer
Test organisms	1. *Aspergillus niger* ATCC 16404 2. *Candida albicans* ATCC 10231 3 *Pseudomonas aeruginosa* ATCC 19429 4 *Staphylococcus aureus* ATCC 6538 Additional organisms, those likely to be found in manufacture and use	1. As BP 2. As BP 3 *Pseudomonas aeruginosa* ATCC 9027 4. As BP 5. *Escherichia coli* ATCC 8739 Additional organisms, those likely to be introduced in use
Test of inoculated samples	0, 6, 24, and 48 hr, 7, 14, and 28 days	0, 7, 14, 21, and 28 days
Criteria of acceptability	Vary according to product. bacteria — not less than 10^3 reduction in population in 6–48 hr, according to product, with strict limits on subsequent growth; molds and yeasts — not less than 10^2 reduction in population in 7–14 days according to product, with strict limits on subsequent growth	Standard for products 1–4. bacteria — not more than 0.1% of original population surviving by day 14 and no further increase up to day 28. molds and yeasts — no increase in population in 28 days

ban the use of mercury-containing compounds in pharmaceutical formulations.

Last in this roll of toxicity reports, *benzethonium chloride*, a quaternary ammonium antimicrobial agent used very slightly in vaccines and ophthalmics, has been reported to be a relatively weak carcinogen [29]

Testing for Preservative Effectiveness

The *United States Pharmacopeia* and *British Pharmacopoeia* contain official testing methodology for evaluating the effectiveness of antimicrobial agents. Those tests are designed to measure the ability of the antimicrobial preservative system in a multiple-dose parenteral, otic, nasal, or ophthalmic product to reduce or destroy a large inoculum of specified microorganisms The *USP* and *BP* tests differ in their approaches to and in details and interpretations of preservative efficacy testing; a summary comparison of the two tests is provided in Table VII, and discussion of the significant and troublesome aspects of preservative efficacy testing follows.

Test organisms *USP* requires five test organisms — three bacterial, one yeast (*Candida albicans*), and one mold spore (*Aspergillus niger*). One of the bacterial challenges is gram-positive (*Staphylococcus aureus*), the other two are gram-negative. *USP* allows the option of using additional microbial challenges in the test if such microorganisms represent contaminants likely to be introduced during use of the product

These test organisms have not been changed since the preservative efficacy test first appeared in *USP XVIII* in 1970 They were selected on the basis of their known association with product contamination, relatively high level of resistance to antimicrobial preservatives, opportunistic contamination capacity, good adaptability, and relatively simple nutritional demands.[30]

BP requires only four challenge organisms (excluding *E. coli*) and uses a different strain of *P. aeruginosa*. Both compendia, however, require a test inoculum of microorganisms to be sufficient to achieve a final product concentration of approximately 10^6 organisms per milliliter or per gram. This level has been criticized as being unrealistically high, but many parenteral manufacturers actually use inoculations containing loads as high as 10^4 organisms per milliliter or per gram.

Pseudomonas probably is the most dangerous contaminant in parenteral products not only because of its adverse physiologic effects but also because of its enzymatic richness, which enables these microorganisms to degrade and/or destroy antimicrobial agents.

Preparation of inoculum and media used Soybean-Casein Digest Agar Medium is used to cultivate bacteria, while Sabouraud Dextrose Agar Medium is used to cultivate yeasts and molds. Microbial inocula are prepared according to similar procedures in *USP* and *BP*, with the exception of the washing and diluting agent *USP* calls for sterile saline TS, while *BP* requires 0.1% peptone water.

Test procedures. *USP* and *BP* procedures are identical regarding the size of the test inoculum, the use of one total product container for each test organism, a maximum 28-day storage of the inoculated containers, room temperature storage, the use of the plate count procedure for determining the number of surviving organisms after each sampling time, and the use of suitable controls The major difference between the *USP* and *BP* procedures is the time of sampling. *USP* requires the taking of weekly samples from the inoculated product, that

is, at 0, 7, 14, 21, and 28 days. *BP* sampling times are quite different: containers are to be sampled at 6, 24, and 48 hr after inoculation and then at 7, 14, and 28 days.

Interpretation. Criteria for accepting the effectiveness of the preservative system are controversially different between the two official methods. *USP* is the more liberal, as may be seen in Table V: *USP* requires a 10^3 reduction in bacterial concentrations by day 14 while *BP* requires the same degree of reduction in only 6 hr. *BP* also has stricter requirements for microbial recovery, or the lack thereof, for bacteria at 24 hr and molds and yeasts after 7 days.

On the one hand, the *BP* test requires a parenteral preservative system to be bactericidal, thus forcing a manufacturer to use upper limit levels of antimicrobial agents in the product formulation. On the other hand, the *USP* method may be too liberal for certain multiple-dose products, such as ophthalmic solutions in which rapid destruction of ingressing microorganisms is vitally desirable.

The development pharmacist must be aware of these important differences between *USP* and *BP* requirements for preservative efficacy evaluation. This is especially essential if the manufacturer plans to register and market the parenteral product in the countries regulated by the British Health and Social Security Department

Accelerated Preservative Efficacy Evaluation Tests

The official compendial preservative tests cannot be replaced as final product evaluation tests for accepting or rejecting a preservative system. In the development stages of a parenteral product formulation, however, there is the need to assess the efficacy of various preservative system possibilities rapidly and economically. Several such screening methods have been described in the literature [26-29]

In a recent study, Akers et al. evaluated the efficacy of 14 preservative systems in insulin solutions against *S. aureus*,[30] using the D-value method first proposed by Orth et al.[26] D values, calculated from linear regression analysis of microbial concentration versus time plots, can be obtained in a relatively short period of time. These values are useful in comparing various preservative systems and in determining which systems deserve further evaluation.

Summary

Selecting the optimal antimicrobial preservative system for parenteral and ophthalmic products is an extremely important responsibility of a development scientist. Limitations that handicap the scientist include the paucity of acceptable parenteral preservative agents, stability and toxicity problems, and difficulties in proper evaluation of preservative activity. It is hoped that this review will serve to help the formulator select and qualify the best preservative system for his or her parenteral formulation.

Acknowledgment

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REVIEW

Excipient–Drug Interactions in Parenteral Formulations

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ABSTRACT: Excipients are added to parenteral formulations to enhance or maintain active ingredient solubility (solubilizers) and/or stability (buffers, antioxidants, chelating agents, cryo- and lyoprotectants). Excipients also are important in parenteral formulations to assure safety (antimicrobial preservatives), minimize pain and irritation upon injection (tonicity agents), and control or prolong drug delivery (polymers). These are all examples of positive or synergistic interactions between excipients and drugs. However, excipients may also produce negative effects such as loss of drug solubility, activity, and/or stability. This review article will highlight documented interactions, both synergistic and antagonistic, between excipients and drugs in parenteral formulations. The reader will gain better understanding and appreciation of the implications of adding formulation ingredients to parenteral drug products. J Pharm Sci 91:2283–2300, 2002

Keywords: parenteral; excipients; formulation; stabilizers; solubilizers; antimicrobial preservatives; packaging

INTRODUCTION

Well-referenced and useful publications are available listing every formulation component in all marketed parenteral drug products[1–5] (Food and Drug Administration web site[a]). The information in these publications has been invaluable to parenteral formulation scientists developing soluble, stable, resuspendable, manufacturable, and deliverable parenteral dosage forms. Formulation component precedence takes on high stature in the sterile product world because of significant toxicological and regulatory concerns. In other words, it is usually better to use a component that has a track record of relatively safe use in injectables and is likely not to raise concerns on the part of regulatory reviewers.

[a]www.fda.gov/cder/drug/iig/default.htm

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This review article will not cover all excipients used in parenteral formulations because the aforementioned publications already do so. What this review article presents are examples of synergistic and antagonist interactions that have been reported for excipients used in parenteral formulations. Although extensive, this review will not be exhaustive in the effort to cite all published references on parenteral drug–excipient interactions. Pharmaceutical Excipients 2000[6] was a very helpful text in obtaining valuable information about drug–excipient interactions and compatibilities.

When one studies stability and compatibility issues in parenteral drug formulation, the packaging system also must be considered. Potential interactions between excipients and rubber closures in finished products are as much a concern as interactions between excipients and drugs. Therefore, some drug–sterile packaging interactions will be covered in this article.

Although this article will focus on chemical and physical compatibilities of drugs and excipients used as injectable products, readers must also be

aware of the potential for any excipient and drug, either alone or in combination, when injected intravenously to cause certain problems. As Yalkowsky et al.[7] pointed out, some formulation-related problems associated with intravenous drug delivery include hemolysis, precipitation, phlebitis, and pain. Therefore, as scientists develop sterile product formulations, not only must they be concerned with physical and chemical interactions that may occur *in vitro*, but they must also be concerned with the potential for formulation-related problems occurring *in vivo*.

Drug–excipient interactions are studied in two basic ways. One is to perform traditional preformulation studies using full factorial or Plackett Burman type of experimental designs. A good example of this approach for parenteral formulation development is a preformulation study published by Peswani and Lalla.[8] Analytical methods such as differential scanning calorimetry (DSC),[9] isothermal microcalorimetry,[10,11] and fourier transform infrared (FT-IR) spectroscopy[12] are excellent tools for predicting drug–excipient interactions. The other approach for studying drug–excipient interactions is to conduct both short-term and long-term stability studies on various formulations of the drug and measure both chemical stability (usually by chromatographic techniques) and physical stability (e.g., by microscopic, electronic particle analysis, and circular dichroism techniques).

This review is organized according to major functions of parenteral excipients (solubilization, stabilization, preservation, and drug delivery aids). Several excipients serve more than one function [e.g., polyvinylpyrrolidone (PVP) as a complexing agent and as a freeze-drying bulking agent], so such excipients may be referenced in more than one segment of the article.

Table 1 lists all the major pharmaceutical excipients used in parenteral formulations. Table 2 provides a listing of lesser-used excipients that are found in 1–2 commercial parenteral formulations. References[1–5] provide much greater detail about the specifics of these excipients (e.g., concentration) and the products in which they are components (e.g., brand names, manufacturer).

Solubility Effects

Many parenteral formulations require additives, either solvent or solute excipient, to increase and/or maintain solubility of the active ingredient in the solution. Sweetana and Akers[13] summarized seven basic approaches for solubilization of parenteral drugs as follows:

1. Salt formation
2. pH adjustment
3. Use of co-solvents
4. Use of surface-active agents
5. Use of complexation agents
6. Change formulation from solution to a dispersed system, oily solution, or a more complex formulation such as a microemulsion or liposome
7. "Heroic" approaches involving the use ofnon-commercially approved types and/or concentrations of solvents or excipients

This section will highlight some of the interactions between drugs and solubilizing agents, focusing on co-solvents, surfactants, suspending and emulsifying agents, complexation agents, and oils or lipids. Some examples of unpredicted interactions of excipients and drugs to enhance drug solubility are listed in Table 3.

Co-Solvents

There are approximately 20 different co-solvent agents used in approved parenteral products. However, the most commonly used co-solvents in parenteral formulations are ethanol, glycerin, propylene glycol, sorbitol, polyethylene glycol (both 300 and 400), dimethylacetamide, Cremophor EL, and *N*-methyl-2-pyrrolidone.

Glycols are widely used solubilizing agents, but can cause some stability or compatibility problems. Glycerol is used not only as a co-solvent for improving solubility of poorly water-soluble drugs, but also as a tonicity-adjusting agent (e.g., in insulin formulations). In freeze-dried formulations, glycerol can serve as a plasticizer, lowering the glass transition temperature of the product without the significant change in water content or activity.[14] In certain formulations containing unstable peptides, the presence of glycerol will increase the mobility of the freeze-dried formulation matrix, leading to peptide deamidation. Sorbitol has been reported to increase the degradation rate of penicillins in neutral and aqueous solutions.[15] On a more positive note, propylene glycol will potentiate the antimicrobial activity of the parabens in the presence of nonionic surfactants and prevents the interaction of methylparaben and polysorbate 80.[16]

Table 1. A Listing of Major Excipients Used in Sterile Product Formulations (Both Commercial and Developmental)

Solvent systems
Co-solvents
Propylene glycol
Glycerin
Ethanol
Polyethylene glycol (300 and 400)
Sorbitol
Dimethylacetamide
Cremophor EL
Oils
Sesame
Soybean
Corn
Castor
Cottonseed
Peanut
Arachis
Ethyl oleate
Isopropyl myristate
Glycofurol
Petrolatum
Solubilization agents
Co-solvents
See above
Surface-active agents
Polyoxyethylene sorbitan monooleate (Tween 80)
Sorbitan monooleate
Polyoxyethylene sorbitan monolaurate (Tween 20)
Lecithin
Polyoxyethylene–polyoxypropylene copolymers (Pluronics®)
Complexation agents
Hydroxypropyl-β-cyclodextrin
Sulfobutylether-β-cyclodextrin (Captisol®)
Polyvinylpyrrolidone
Amino acids (arginine, lysine, histidine)
Stabilization agents
Buffers
Acetate
Citrate
Tartrate
Phosphate
Triethanolamine (TRIS)
Antioxidants
Ascorbic acid
Acetylcysteine
Sulfurous acid salts (bisulfite, metabisulfite)
Monothioglyercol
Chelating agents
Ethylenediaminetetraacetic acid (EDTA)
Sodium citrate
Cryo- and lyoprotectants and bulking agents
Mannitol
Glycine
Sucrose
Lactose
Trehalose
Dextran
Povidone
Sorbitol
Competitive binding agents
Serum albumin
Heta-starch
Tonicity-adjusting agents
Sodium chloride
Glycerin
Mannitol
Dextrose
Antimicrobial preservative agents
Phenol
Meta-cresol
Benzyl alcohol
Parabens (methyl, propyl, butyl)
Benzalkonium chloride
Chlorobutanol
Thimerosal
Phenylmercuric salts (acetate, borate, nitrate)
Delivery polymers
See Table 6

Co-solvents are known to cause hemolysis. In a study conducted by Fuet et al.[17] comparing the hemolytic effects, both *in vitro* and *in vivo*, of a variety of co-solvents (ethanol, propylene glycol, polyethylene glycol, dimethylisosorbide, and dimethylacetamide), complexing agents (nicotinamide), and surfactants (Pluronic L64 and emulphor EL-719), solutions most prone to elicit a hemolytic response were those containing propylene glycol, dimethylisosorbide, and nicotinamide). However, these authors found that the hemolytic effects of propylene glycol can be alleviated by the addition of either a tonicifying agent or polyethylene glycol 400.

Cremophor EL (polyoxyl 35 castor oil) has been approved as a solvent in commercial injectable dosage forms containing paclitaxel, diazepam, propanidid, and alfaxalone. It is compatible with many organic solvents and aqueous solutions. However, compounds containing phenolic hydroxyl groups may cause precipitation of Cremophor EL.

Surfactants

Surfactants serve a variety of very important functions in parenteral formulations. Among the most important are stabilizing proteins against aggregation. Tween 20 (polyoxyethylene sorbitan monolaurate) was shown to greatly reduce the

Table 2. Examples of Special or Uncommon Excipients Used in Injectable Drug Products

Excipient	Product	Manufacturer
Acacia	Tuberculin Old Test (ID)	Lederle
Acetone sodium	Talwin (IM)	Sanofi Winthrop
Aluminum monostearate	Solganal	Schering
Benzenesulfonic acid	Tracrium	Glaxo Smith Kline
Benzyl benzoate	Depo-testesterone	Pharmacia
Cyclodextrin (alpha)	Alprostadil	Schwarz
Diethanolamine	Bactrim	Roche
Desoxycholate sodium	Fungizone	Bristol Myers Squibb
Formaldehyde	Some vaccines	Lederle, Connaught, Merck
Gelatin, hydrolyzed	Some vaccines	Merck
Gelatin, purified	Lupron Depot	TAP
Hydroxypropyl-β-cyclodextrin	Itraconazole	Janssen
Imidazole	Kogenate	Bayer
Monoethanolamine	Terramycin (IM)	Roerig
N,N-dimethylacetamide	Vuman	Bristol Myers Squibb
	Busulfan	Orphan
Polyoxyethylated fatty acid	AquaMephyton	Merck
PEG 40 castor oil	Monistat	Janssen
PEG 60 castor oil	Prograf	Fujisawa
Sodium lauryl sulfate	Proleukin	Cetus
Sulfobutylether-β-cyclodextrin	Ziprasidone mesylate	Pfizer
Triacetin	Prepidil Gel (ICV)	Pharmacia

rate of formation of insoluble aggregates of recombinant human factor XIII caused by both freeze thawing and agitation stresses[18] (Fig. 1). Maximum protection occurs at concentrations close to the critical micelle concentration of Tween 20, independent of initial protein concentration. In another report, Tween 20 at a 1% (w/v) concentration caused precipitation of a relatively hydrophobic protein (*Humicola lanuginosa lipase*) by inducing non-native aggregates.[19]

Tween 80 is well known to protect proteins against surface-induced denaturation.[20] Tween 80 was demonstrated to reduce hemoglobin aggregation in solution by preventing the protein from reaching the air–liquid interface or the liquid–surface interfaces.[21] Polyoxyethylene surfactants such as Tween 80 can form peroxide impurities after long-term storage. Knepp et al.[22] concluded that Tween 80 and other nonionic polyether surfactants undergo oxidation during bulk material storage and subsequent use and the resultant alkyl hydroperoxides formed can contribute to the degradation of proteins. In such formulations, they further reported that thiols such as cysteine, glutathione, and thioglycerol were most effective in stabilizing protein formulations containing peroxide-forming nonionic surfactants.

The nonionic surfactant octoxynol 40 (ethoxylated alkyl phenol, Igepal CA897, GAF), was found to solubilize an otherwise insoluble complex of a nonsteroidal anti-inflammatory drug and a quaternary ammonium antimicrobial preservative

Table 3. Examples of Esoteric Excipients Used as Solubilizers

Generic Name	Brand Name	Manufacturer	Excipient
Ciprofloxacin	Cipro IV	Bayer	Lactic acid
Doxorubicin HCl	Adriamycin RDF	Pharmacia	Methyl paraben
Ergotrate Maleate	Ergonovine maleate	Lilly	Ethyl lactate
Polyestradiol phosphate	Estradurin	Wyeth	Niacinamide
Zomepirac	Zomax	McNeil	Tromethamine[105]

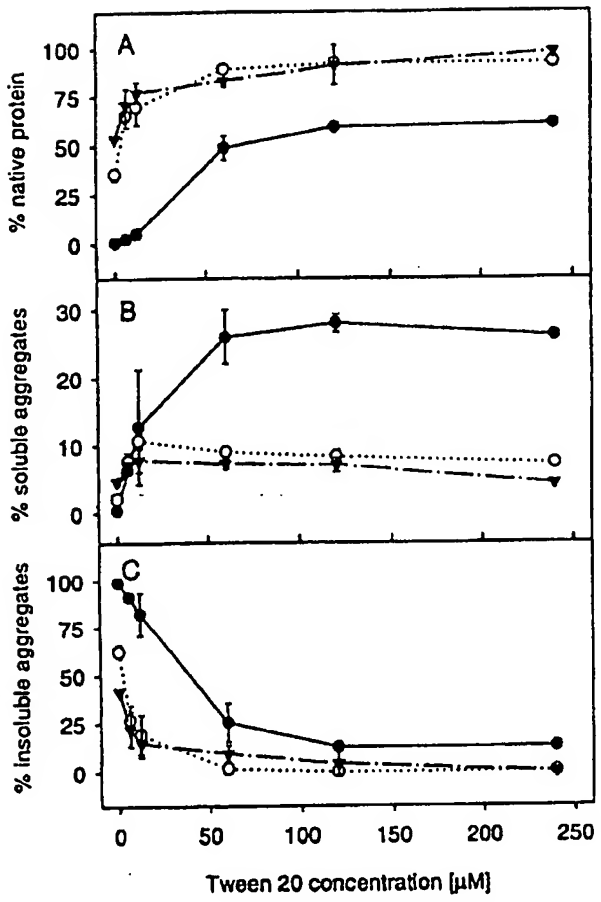


Figure 1. Recovery of native rFXIII (A) and formation of soluble (B) and insoluble (C) aggregates after 10 freeze–thaw cycles of 1 mg/mL (●), 5 mg/mL (○), and 10 mg/mL (▼) as a function of Tween 20. (From Krielgaard et al., J Pharm Sci, 87, 1593–1603, © 1998 John Wiley & Sons, Inc., reproduced with permission.)

mixture in an ophthalmic formulation.[23] This is a rather unique drug–excipient interaction in which the interaction of the excipient involves not only a drug, but also a drug–preservative combination that is otherwise incompatible.

Complexing and Dispersing Agents

Cyclodextrins have emerged as very effective additive compounds for solubilizing hydrophobic drugs. In the parenteral dosage form area, modified cyclodextrins, such as hydroxylpropyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin have been reported to solubilize and stabilize many injectable drugs, including dexamethasone, estradiol, interleukin-2, and other proteins and peptides[24] without apparent compatability problems.[25] There are still only a few approved products worldwide that contain cyclodextrins (see Table 2). However, based on the literature and scientific meeting presentations, there will be a higher number of cyclodextrin-containing injectable formulations in the future.

The only reports of incompatibilities with cyclodextrins involve certain antimicrobial preservatives, primarily parabens.[26,27] One preliminary report described both sulfobutylether-β-cyclodextrin and hydroxylpropyl-β-cyclodextrin accelerating the degradation of an unidentified water-soluble drug to its insoluble degradant form.[28] The authors concluded that both the type and degree of substitution of the proximal hydroxyl groups in the cyclodextrin cavity will influence the potential for cyclodextrin additives to accelerate chemical degradation of drugs. As cyclodextrins become more prominent in injectable drug product development, there likely will be more reports of incompatibilities along with the expected reports describing solubility and stability enhancements.

Cyclodextrin-containing formulations (either 0.1 M sulfobutylether-β-cyclodextrin or 0.1 M hydroxylpropyl-β-cyclodextrin) were shown to cause less damage to venous epithelial cells at the site of injection compared with formulations containing organic co-solvents.[29] PVP (povidone) is a generally compatible polymeric excipient. However, it can form molecular adducts (a positive reaction with respect to iodine therapy topically) and will complex with some preservatives such as thimerosal.[6] Lecithin is a commonly used emulsifying and stabilizing agent in intramuscular and intravenous injections, primarily the intravenous fatty or lipid emulsions used in parenteral nutrition. Lecithin also is a component of some liposomal formulations. Polaxamers (e.g., Poloxamer 188, BP) are nonionic polyoxyethylene–polyoxypropylene copolymers used as emulsifying agents in intravenous fat emulsions. They have also been used in several patented protein formulations as stabilizers and sustained release injectables in development as solubilizing and stabilizing agents.[30] Polaxamers, like the polysorbates, can form peroxide impurities over time and are incompatible with antimicrobial preservatives such as phenol and paraben.

Oils/Lipids

Many commercially available parenteral products contain lipophilic or oleaginous solvents. Examples of injectable lipid solvents include ethyl

oleate, isopropyl myrsistate, glycofurol, and fixed vegetable oils (e.g., peanut, corn, cottonseed, sesame, soybean, castor, arachis). There are several oily injectable solutions and suspensions used as sustained-release formulations (Clopixol®, Haldol Decanoate®, Deca-Durabolin®, Modecate®, Depixol®, and others).[31] Oils are generally compatible with lipophilic drugs and excipients. However, formulation scientists must be aware of the potential for oils to be absorbed by rubber closure materials.[32,33] Oils can contain unacceptable impurities (e.g., gossypol in cottonseed oil, saturated fatty materials, unsaponifiable materials, other organic residuals), but the United States Pharmacopeia and other compendia specify limits on these impurities.

Highly purified sesame oil was found to improve the long-term stability of lidocaine.[34] Lidocaine in sesame oil that was not purified was degraded and formed crystals because of oxidation products such as hydroperoxides and impurities such as mono- and di-glycerides, free fatty acids, plant sterols, and the colorants chlorophyll and carotene).

Soybean oil is the preferred oil in parenteral fatty emulsions. Soybean oil emulsions have been studied extensively and have been found to be incompatible with calcium chloride, calcium gluconate, magnesium chloride, phenytoin sodium, tetracycline hydrochloride, and potentially many other drug substances and intravenous infusion solutions.[35]

Petrolatum is a commonly used ointment base for topical ophthalmic ointments. There are no known incompatibilities with petrolatum.[36]

Stability Effects

This section highlights both classical and more recent publications that report on both positive and negative effects of excipients on drug stability in parenteral formulations. Examples of some esoteric effects (the stabilization of the drug by the excipient was not predicted or expected) of excipients stabilizing certain drugs are given in Table 4.

Table 4. Esoteric Examples of Excipient Stabilizers

Drug or Brand Name	Stabilizing Agent
Dexamethasone acetate	Creatine or creatinine
Cardiotec	Gamma cyclodextrin
Albumin	Sodium caprylate
Stelazine/compazine	Sodium saccharine

Buffers

Buffer components in parenteral formulations can cause stability problems. Phosphate buffer, particularly the dibasic phosphate anion, serves as a nucleophile that can attack electrophilic centers like ester or amide carbonyl groups or polarized carbon-nitrogen double bonds.[37]

Hasegawa et al.[38–42] published several articles describing the use of pharmaceutical phosphate buffer solutions in the presence of calcium and/or aluminum by the use of EDTA in pH ranges of 5 to 9, carboxylic acids such as citric acid and maleic acid in acidic to neutral solutions, and pyrophosphate and lysine hydrochloride in alkaline solutions. Of course, the best approach is to eliminate metal contaminants in solutions or additives by techniques such as ion exchange, but often this is not practical.

Zhu et al.[43] presented a poster describing a study in which an unstable drug needed to be buffer stabilized at pH 3. Four buffer systems (citrate, glycinate, maleate, and tartrate) were studied using the same pH; only one buffer (glycinate) did not catalyze drug degradation (see Fig. 2). The authors did not speculate on why glycinate buffer did not catalyze the degradation of the drug although low concentrations (0.1 M) of all buffers would be acceptable. In a similar study, Nakamura et al.[44] found that

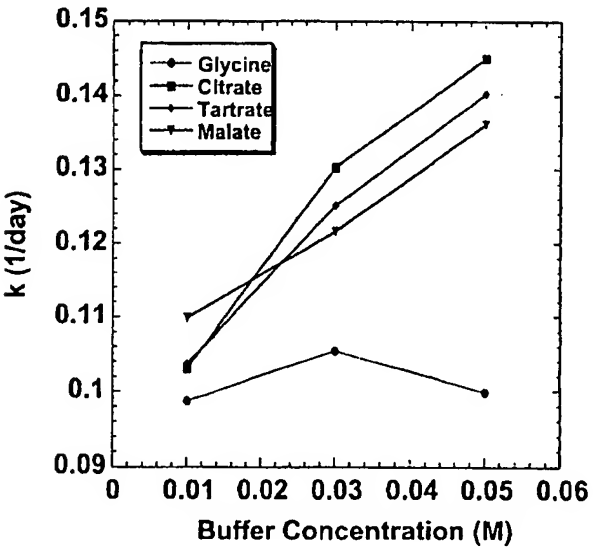


Figure 2. Rate of hydrolysis of GW280430 (0.2 mg/mL) as a function of buffer type and concentration at 60°C. (From Zhu, Merserve, & Floyd, Drug Dev Ind Pharm, 28, 135–142, © 2002 Marcel Dekker, Inc., reproduced with permission.)

Table 5. Stability Data for Minodronic Acid in Different Buffer Systems[44]

Buffer	pH	Potency (%) After 4 Weeks at 60°C	Number of Particles >2 μm in 100 mM Buffer After 4 Weeks at 60°C
Glycine HCl	3.0	78.1	1000–9999
	5.1	95.5	>10,000
Citrate	3.0	99.5	0–99
	5.0	101.8	0–99
Succinate	3.1	82.4	1000–9999
	5.0	94.4	>10,000
Acetate	3.1	74.8	100–999
	5.0	88.5	1000–9999
Tartrate	3.0	93.4	0–99
	5.0	95.7	0–99
Lactate	3.1	86.0	100–999
	4.4	93.9	1000–9999
Maleate	3.1	87.3	1000–9999
	5.0	80.1	>10,000

citrate and tartrate buffers maintained both chemical and physical (fewer particles) stability of minodronic acid in a parenteral aqueous solution whereas glycine, succinate, acetate, lactate, and maleate buffers did not (see Table 5). Li et al.[45] compared the stability of tezacitabine in three buffer systems (phosphate, glycine, and TRIS) and found phosphate to be superior. Higher concentrations of phosphate also improved drug stability (see Fig. 3a and b).

Tris buffer, when used in a peptide formulation and stored at 70°C, will degrade to liberate formaldehyde.[46] Although this was not seen at lower temperatures, formulators need to be aware of this possible instability when using this common biological buffer.

Tris buffer will form a stable complex with *N*-nitrosourea anticancer agents and retard the degradation of these agents.[47] However, Tris buffer will degrade 5-fluorouracil, causing the formation

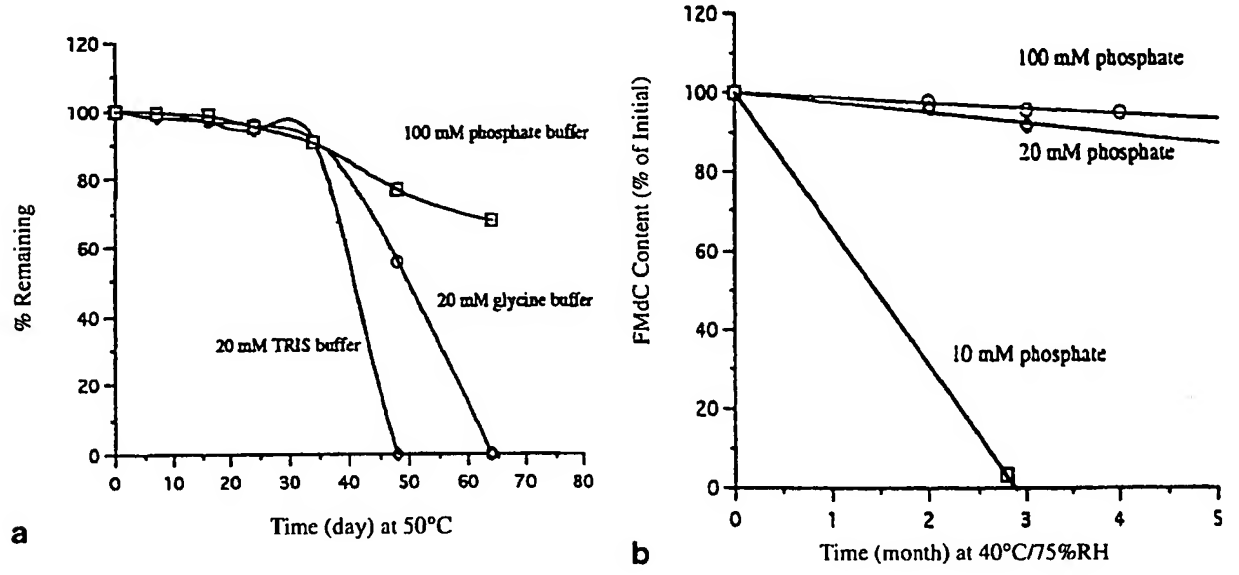


Figure 3. (a) Effect of buffer on stability of FMdC solution at 10 mg/mL. (b) Effect of phosphate buffer concentration on stability of FMdC solution at 10 mg/mL. (From Li, Chang, Pan, & Jones, AAPS Pharm Sci, (S) 3, © AAPS Denver, reproduced with permission.)

of two degradation products that can cause serious-to-lethal cardiotoxicities.[48]

Sometimes the cationic species in a buffer system matters. Sarciaux et al.[49] found that a sodium phosphate buffer system consistently resulted in more turbid reconstituted solutions of bovine immunoglobulin (IgG) than a potassium phosphate buffer system at the same concentration. The authors believed that this effect was not attributable to a pH shift sometimes seen during freezing as a result of crystallization of sodium phosphate. This is because sodium chloride-containing formulations also showed substantially higher levels of aggregation compared with potassium chloride-containing formulations. Bovine IgG will denature at the ice/freeze-concentrate interface that is irreversible after freeze-drying and reconstitution. This ice/freeze-concentrate interfacial denaturation is dependent on the amount or percentage of protein residing at the ice/freeze-concentrate interface and the surface area of the freeze-dried solid. Formulations containing sodium salts showed a higher surface area of dried solids than formulations containing potassium salts. The higher the surface area, the more drug is exposed to the interfacial area, resulting in a higher degree of denaturation and resultant aggregation. When phosphate buffers are frozen, selective precipitation of the less-soluble buffer component (dibasic sodium phosphate) and subsequent pH shift may induce protein denaturation.[50] In the case of monomeric and tetrameric β-galactosidase, both sodium and potassium phosphate buffers caused significant secondary structural perturbations, greater for sodium phosphate samples. The addition of sucrose was able to minimize this freeze-dried denaturation in phosphate buffers, even if there remained large-scale changes in solution pH during freezing.

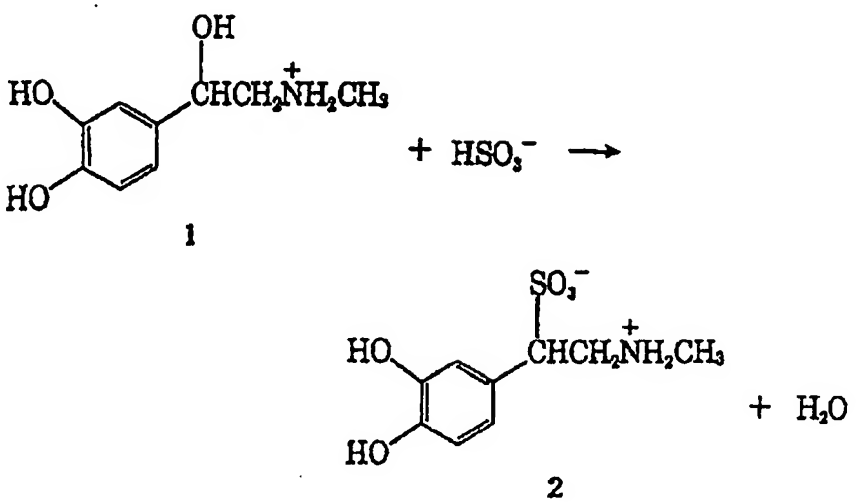
Histidine was used as a buffer system in an experimental formulation containing humanized IgG2 monoclonal antibody.[51] Histidine underwent oxidation and the oxidation products caused a significant loss of potency of the monoclonal antibody. The antibody also degraded in citrate buffer, although not as much as in histidine buffer. Histidine buffer oxidizes in the presence of peroxides and the source of peroxides in these formulations presumably originated from Tween 80 also present. Nitrogen overlay inhibited the histidine buffer oxidation and enhanced antibody potency.

Antioxidants

Ascorbic acid has been reported to be incompatible with certain drugs such as penicillin G.[52] However, this is not a direct incompatability of two organic molecules, but rather an incompability caused by the pH effects of ascorbic acid,[53] as specified by Stella.[37]

Sodium bisulfite and sodium metabisulfite are strongly nucleophilic antioxidants capable of catalyzing drug degradation.[37] The well-known interaction of bisulfite and epinephrine leads to degradation of epinephrine. (Scheme 1)

Epinephrine reacts with bisulfite to form the sulfonic acid derivative.[54] However, the addition of sodium borate complexes the parahydroxybenzyl



Scheme 1.

derivatives and prevents the reaction between epinephrine and bisulfite.[55] Zinc, copper, and iron also will not catalyze any reaction between epinephrine and bisulfite.[56] Interestingly, aluminum (III) will catalyze epinephrine degradation via a complexation effect not seen with these other metal ions.[56]

Sodium bisulfite also has been reported to dehalogenate uracil-type molecules[57] and cause ester hydrolysis.[58] Bisulfite will react with physostigmine in aqueous solutions rapidly and reversibly, which is dependent both on the total bisulfite added and pH.[59] Bisulfite will attack physostigmine on the optically active carbon-10a,[60] which is reversed if the mixture is diluted and pH adjusted to values greater than pH 6. This pH dependency suggested that the reaction involved both SO_3^{2-} and HSO_3^- reacting with the protonated form of physostigmine.

Sodium metabisulfite inactivates cisplatin in solution[61] and is incompatible in ophthalmic solutions containing phenylmercuric acetate, especially when autoclaved.[62] If dextrose and sodium metabisulfite are combined in aqueous solution, metabisulfite stability declines.[63]

Ascorbic acid is a frequently used antioxidant in parenteral formulations. Formulators must be aware that ascorbic acid generally is incompatible with alkaline solutes, heavy metals, phenylephrine hydrochloride, pyrilamine maleate, salicylamide, sodium nitrite, sodium salicylate, and theobromine salicylate.[64] Kerwin et al.[65] found that ascorbate ion in sufficient concentrations reacted with oxygen producing superoxide that in turn caused chemical modification and aggregation of recombinant deoxy hemoglobin. Lower levels of ascorbate and oxygen and lower solution pH combined to eliminate this problem.

Edetic acid and its salts are used as metal chelating agents to aid in stabilization of drugs sensitive to metal-catalyzed oxidation and/or photolysis. They also can serve to enhance antimicrobial activity of formulations. Edetate salts are incompatible with zinc insulin, thimersosal, amphotericin, and hydralazine hydrochloride.[6]

Bulking Agents and Lyoprotectants

Several mechanisms have been proposed to explain why excipients serve as cryo- or lyo protectants. The most widely accepted mechanism to explain the action of cryoprotection is the preferential exclusion mechanism.[66] Excipients that will stabilize proteins against the effects of freezing do so by not associating with the surface of the protein. Such excipients actually increase the surface tension of water and induce preferential hydration of the protein. Examples of solutes that serve as cryoprotectants by this mechanism include amino acids, polyols, sugars, and polyethylene glycol.

For lyoprotection, that is, stabilization of proteins during the drying stages of freeze drying and during storage in the dry state, two mechanisms have been generally accepted. One is the water-substitute hypothesis[67] and the other is the vitrification hypothesis.[68] Both are legitimate theories, but both also have exceptions, i.e., neither fully explain the stabilization of proteins by excipients during dehydration and dry storage.[69] The water-substitute hypothesis states that a good stabilizer is one that hydrogen bonds to the protein just as water would do were it present and, therefore, serves as a water substitute. Sugars are good water substitutes[b] which is why many freeze-dried protein formulations contain sucrose or trehalose.

The vitrification hypothesis states that excipients that remain amorphous (glass formers) form a glassy matrix with the protein with the matrix serving as a stabilizer. Acceptance in this hypothesis requires formulators to determine glass transition temperatures of formulations to be freeze dried and to develop freeze-dry cycles that maintain drying temperatures below the glass transition temperature. Studies have been published showing that excipient stabilizers that crystallized during storage caused degradation (typically aggregation and loss of potency) of the protein.[70–72]

Freeze-dried formulations typically contain one or more of the following bulking agents: mannitol, lactose, sucrose, trehalose, dextran 40, and povidone. These excipients may also serve as cryo- and/or lyoprotectants in protein formulations. Fakes et al.[73] studied these bulking agents for moisture sorption behavior before and after freeze drying. Moisture uptake certainly can affect drug stability in the freeze-dried state, particularly with proteins. They reported the following observations for each excipient:

[b]It may at first appear contradictory that sugars can serve both as cryoprotectants because of being excluded from the surface of the protein and as lyoprotectants that hydrogen bond to the protein. However, keep in mind that the excluded solute concept involves a frozen aqueous system whereas the water-substitute concept occurs in a dry system.

Mannitol
Crystalline and nonhygroscopic both before and after freeze drying
Total moisture contents of 0.1 to 0.3% w/w between 10 and 60% relative humidity (RH)

Lactose
Crystalline and nonhygroscopic before lyophilization
Moisture content 0.86% before lyophilization
Amorphous after lyophilization with moisture content of 1.6%
Absored moisture rapidly upon exposure to high RH
Converted to crystalline form at 55% RH after absorption of 10% moisture
Desorption of all moisture sorbed by the amorphous form

Sucrose
Crystalline and nonhygroscopic before lyophilization
Moisture content 0.15% before lyophilization
Amorphous after lyophilization with moisture content of 2.5%
Absorbed moisture rapidly upon exposure to high RH
Converted to crystalline form at 50% RH after absorption of 4.5% moisture
Desorption of all moisture sorbed by the amorphous form

Trehalose
Crystalline and nonhygroscopic before lyophilization
Moisture content 9.2% before lyophilization
Amorphous after lyophilization with moisture content of 1.2%
Absorbed moisture rapidly upon exposure to high RH
Converted to crystalline form at 50% RH after absorption of 10% moisture
Desorption of all moisture sorbed by the amorphous form

Dextran
Amorphous and hygroscopic both before and after freeze drying
Sorbed 10–20% moisture at 50% RH

PVP
Amorphous and hygroscopic both before and after freeze drying
Sorbed 10–20% moisture at 50% RH

When selecting a bulking agent, these properties, particularly the tendency for moisture uptake, must be considered by the formulation scientist in developing an optimally stable freeze-dried formulation.

Several excipients can serve as stabilizers for proteins that are unstable during the drying phases of freeze drying and/or during long-term storage in the dry state. Typically, additives that will crystallize during lyophilization (e.g., mannitol) or will remain amorphous but unable to hydrogen bond to the dried protein (e.g., dextran) are not effective lyoprotectants for proteins. Excipients that will crystallize during freeze drying will also be relatively ineffective as was shown with sucrose in *Humicola lanuginosa* lipase formulations[19] (Fig. 4). However, these authors also reported that sucrose crystallization could be inhibited by decreasing the mass ratio of sucrose to protein and by minimizing the moisture content that serves to decrease the glass transition temperature during storage.

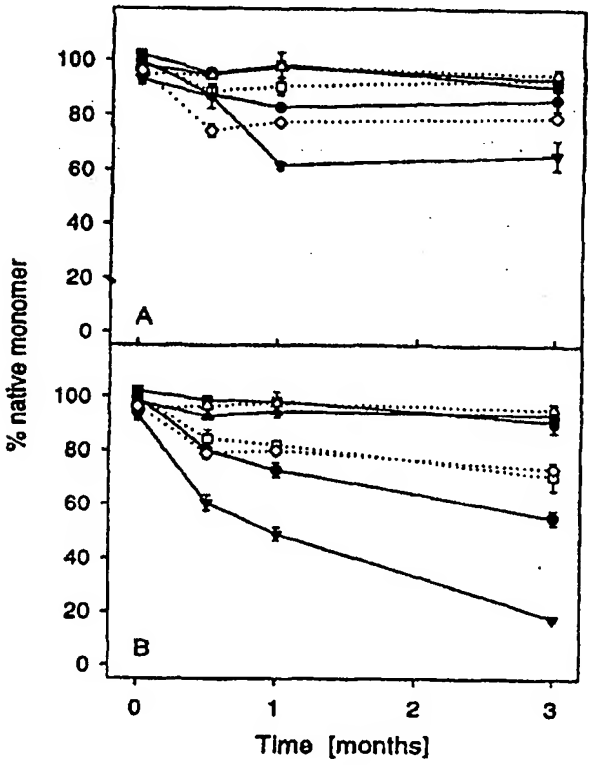


Figure 4. Recovery of native, monomeric HLL as a function of time as determined by size exclusion chromatography. Formulations stored at 40°C (A) or 60°C (B) in the absence of additives (●) or in the presence of 300 mM mannitol (▼), 5% (w/v) dextran (◇), 50 mM trehalose (▲), 300 mM trehalose (△), 50 mM sucrose (■), or 300 mM sucrose (□). (From Krielgaard, Frokjaer, Flink, Randolph, and Carpenter, J Pharm Sci, 88, 281–290, © 1999 John Wiley & Sons, Inc., reproduced with permission.)

The reverse can also be true for certain small molecules. For example, excipients (mannitol or sodium bicarbonate) that promoted the crystallization of cyclophosphamide during freeze drying stabilized the final product whereas excipients that did not allow crystallization (e.g., lactose) destabilized the final product.[74]

Costantino et al.[75] studied the effects of a variety of parenteral excipients on stabilizing human growth hormone in the lyophilized state. Mannitol, sorbitol, methyl α-D-mannopyranoside, lactose, trehalose, and cellobiose all provided significant protection of the protein against aggregation, particularly at levels (131:1 excipient-to-protein molar ratio) to potentially satisfy water-binding sites on the protein in the dried state. At higher excipient-to-protein ratios, mannitol and sorbitol crystallized and were not as effective in stabilizing the protein compared with low levels in which they remained in the amorphous, protein-containing phase.

Reducing sugars may not be as effective as other bulking agents, cryoprotectants, or lyoprotectants because they may potentially react with proteins via the Maillard reaction. For example, glucose will form covalent adducts with side-chain amino acids lysine and arginine of human relaxin.[76] In addition, a significant amount of serine cleavage from the C-terminal of the B-chain of relaxin was formed when glucose was used as the excipient. These reactions did not occur if mannitol and trehalose replaced glucose in the lyophilized formulation.

Lactose will react with primary amines in the well-known Maillard-type condensation reaction to form brown-colored degradation products.[12] Thus, lactose is known to be incompatible with amine-containing compounds such as aminophylline, amphetamines, and amino acids. This reaction occurs more readily with amorphous lactose than crystalline lactose.

Mannitol is probably the most widely used bulking agent in lyophilized formulations because of its many positive properties with respect to crystallinity, high eutectic temperature, and matrix properties. However, some lots of mannitol can contain reducing sugar impurities that were implicated in the oxidative degradation of a peptide in a lyophilized formulation.[77] Mannitol, at or above certain concentrations and volumes in glass vials, is well known to cause vial breakage because of the unique crystallization properties of mannitol-ice during the primary drying states of freeze drying.[78,79]

Other Freeze-Dry Excipients

High-molecular-weight carbohydrates, such as dextran, have higher glass transition temperatures than proteins. Therefore, when mixed with proteins, the overall glass transition temperature presumably can be increased with resultant increases in protein storage stability. Typically, carbohydrates (sucrose, trehalose, or dextran) alone do not result in appreciable increases in storage stability of proteins. However, combinations of dissacharide and polymeric carbohydrates do tend to improve protein storage stability.[80]

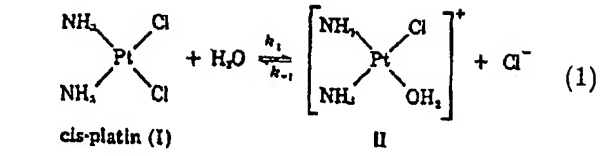
However, singular carbohydrates (sucrose or trehalose at 60 mM) were just as effective in stabilizing a model recombinant humanized monoclonal antibody as combinations of sucrose and mannitol or trehalose and mannitol. Interestingly, with this monoclonal, mannitol alone at 60 mM provided less protection during storage than sucrose or trehalose alone. A specific sugar/protein molar ration was sufficient to provide storage stability for this particular monoclonal antibody.[81,82]

Low-molecular-weight additives, such as osmolytes (*N,N'*-dimethylglycine, trehalose, and sucrose) or salts (sodium chloride, sodium phosphate, ammonium sulfate, and sodium citrate) were found to be highly effective in stabilizing keratinocyte growth factor, both against thermal denaturation and enhancing long-term storage stability.[83]

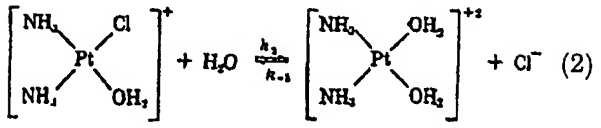
PVP and glycine were found to stabilize lyophilized sodium prasterone sulfate whereas dextran 40 or mannitol did not.[84] PVP and glycine stabilized the pH of the reconstituted solution by neutralizing the acidic degradation product, sodium bisulfate, formed by the hydrolysis of prasterone sulfate. Dextran 40 or mannitol were ineffective because of no buffer capacity. Buffering agents, such as phosphate-citrate buffer and some neutral and basic amino acids (L-arginine, L-lysine, and L-histidine), also stabilized prasterone sulfate. L-cysteine is an example of an amino acid that did not stabilize the drug, presumably because of its weak buffer capacity.

Tonicity Agents

Sodium chloride will significantly stabilize the solution stability of cis-platin because of the presence of chloride ion that shifts the equilibria shown in eqs. 1 and 2 in favor of eq. 1.[85]



(1)



(2)

(Equations 1 and 2 from Hincal, Long, and Repta, J Parenter Sci Technol, 33, 107–116, © 1979 Parenteral Drug Association, reproduced with permission.)

Consequently, the use of other chloride-containing aqueous solutions, such as lactated Ringer's or Ringer's injections, will also stabilize cisplatin. Sodium bicarbonate and alkaline solutions in general will enhance degradation of cis-platin whereas mannitol or dextrose have no effect on the rate or extent of loss of cis-platin.

The potential salting-out effects of marginally soluble drugs and excipients must be considered with any usage of sodium chloride. For example, methylparaben will salt out in the presence of sodium chloride in solution.[86]

Dextrose solutions of various concentrations (5% isotonic) are major intravenous diluents. In intravenous therapy, many drugs and drug products are combined with dextrose solutions for controlled infusion of these drugs. Trissel's book[35] contains compatibility information for all drugs and dextrose as well as all other diluents. Drugs that are known to be incompatible with dextrose include cyanocobalamin, kanamycin sulfate, novobiocin sodium, warfarin sodium, erythromycin gluceptate, B-complex vitamins, and gemcitabine. Formulators wishing to use dextrose as an nonionic tonicity-adjusting agent must be aware of its degradation upon excessive heating[87] and its potential for reacting with amine drugs (browning reaction).

Antimicrobial Preservative Effects

Structurally, most antimicrobial preservative agents are volatile and very reactive with many different types of organic molecules. Literature refers to interactions of various preservatives with drugs, excipients, packaging, and filter materials. Benzalkonium chloride, the most common ophthalmic product preservative system, is well known to adsorb onto various filter membrane surfaces.[88] Benzyl alcohol antimicrobial activity is reduced in the presence of nonionic surfactants, such as polysorbate 80, although this reduction is less than what is seen with hydroxylbenozoate esters (parabens)[89] or quarternary ammonium compounds (benzalkonium chloride).[90] As noted previously, propylene glycol will prevent the incompatibility between paraben and polysorbate 80.[17]

Benzyl alcohol is incompatible with methylcellulose and can be adsorbed by rubber closures composed of natural rubber, neoprene rubber, and butyl rubber.[91] Plastic materials, such as polyethylene or polystyrene, should not be used with solutions containing benzyl alcohol, although plastics such as polyvinyl alcohol and polypropylene are compatible with benzyl alcohol.[92] Plastic materials also will adsorb the parabens although low- and high-density polyethylene containers do not.[93]

Meta-cresol has been reported to be incompatible with chlorpromazine[94] and, like benzyl alcohol and possibly other antimicrobial preservatives, the antimicrobial activity of meta-cresol is reduced in the presence of nonionic surfactants.[6]

Chlorobutanol, a commonly used ophthalmic antimicrobial preservative, will complex with polymers such as polyethylene, carboxymethylcellulose, and polysorbate 80, and will be sorbed by the polymers of plastic vials.[95] The binding (sorption) of chlorobutanol into rubber closures is well known.[96]

Thimerosal, no longer a preferred antimicrobial preservative in newly developed parenteral multi-dose formulations, is still a component of older formulations. Thimerosal is adsorbed by rubber closures,[97] is incompatible with sodium chloride solutions,[98] and is incompatible with a wide range of antioxidants, chelating agents, proteins, and other antimicrobial preservative agents.[6]

Phenylmercuric salts (acetate, borate, nitrate) also are no longer preferred antimicrobial preservatives because of their mercury content. These salts have many incompatibilities including many suspending agents, antioxidants such as sodium metabisulfite, disodium edetate, amino acids, and many rubber and plastic materials.[6]

Any formulator of a protein or peptide dosage form likely has experienced compatibility problems in attempting to develop multi-dose biopharmaceutical products. However, very few articles have been published describing these problems. Lam et al.[82] are among the few who have published their experience with an incompability

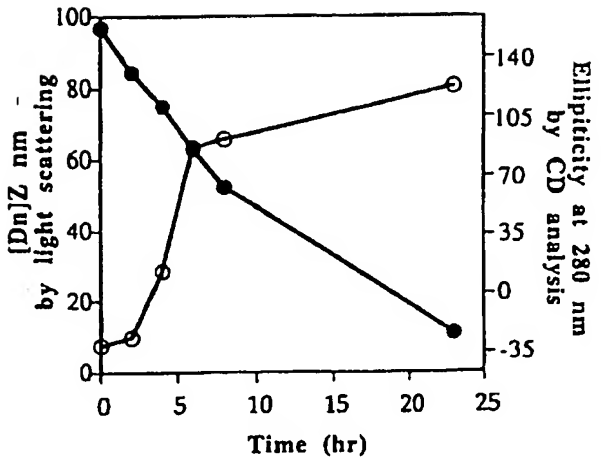


Figure 5. Time course of aggregate formation of 1.0 mg/mL rhIFN-gamma in 5 mM succinate, pH 5.0 in the presence of 0.9% benzyl alcohol as determined by dynamic light scattering (○) and by circular dichroism analysis (●). (From Lam, Patapoff, & Nguyen, Pharm Res, 14, 725–729, © Kluwer Academic Publishers, reproduced with permission.)

between a protein and an antimicrobial preservative. They found that benzyl alcohol caused the aggregation of recombinant human interferon gamma (Fig. 5).

Various preservatives (benzyl alcohol, methylparaben, propylparaben, chlorobutanol, phenol, and m-cresol) were formulated with a humanized monoclonal antibody and tested for compatibility and antimicrobial activity.[99] The protein precipitated in the presence of phenol or m-cresol, but was not adversely affected in the presence of the parabens and low concentrations of chlorobutanol. Benzyl alcohol causes aggregation at high concentrations, but was the most effective antimcrobial preservative agent against bacterial and fungal challenges. However, a combination of methylparaben and chlorobutanol produced a synergistic effect with respect to antibacterial (although no improvement against fungi) activity while still maintaining stability of the protein.

Several marketed protein dosage forms specify that the protein should not be reconstituted with a diluent containing a preservative, presumably because of adverse effects of the preservative on the stability of the protein. Examples include Activase®, Proleukin®, and Leukine®.

Antimicrobial preservatives serve a unique function in insulin formulations.[100] Insulin zinc suspensions (the Lente series) are preserved with methylparaben because phenol will destroy the crystals. However, neutral protamine insulin (NPH) formulations require the use of phenol or phenol derivatives (e.g., meta-cresol) to form the tetragonal oblong crystals characteristic of this widely used prolonged insulin formulation.

Delivery Effects

It is beyond the scope of this review to consider the huge variety of excipient materials used in drug-delivery formulations for sustained and controlled release of injectable drugs (see Table 6). Some polymers used to control drug delivery are discussed herein. The reader is referred to recent publications edited by Sanders and Hendren[101] and Senior and Radomsky[102] for information on excipients used in injectable drug-delivery formulations.

Topical ophthalmic formulations use viscosity-inducing agents such as methylcelluose, hydroxypropylmethylcellulose, and polyvinyl alcohol to enhance drug bioavailablity simply by increasing contact time with the corneal epithelium and retard drug loss via the tearing mechanism. Formulators must recognize that the cellulosic expcipients are generally incompatible with a wide variety of antimicrobial preservatives (e.g., chlorocresol, phenol, cetylpyridinium chloride, and the parabens). Polyvinyl alcohol is less interactive, but at high concentrations ($\geq 5\%$) will precipitate from solutions containing sulfate and phosphate salts.[6]

Carbomers (carbopols) are acrylic acid polymers used in ophthalmic formulations for sustained-release drug delivery.[103] They are incompatible with phenol and potentially other antimicrobial preservative agents, cationic polymers, strong acids, and high levels of electrolytes.[6] Lactide/glycolide homo-polymers, poly(lactic acid) or poly(glycolic acid), or the copolymers, poly(lactic acid-*co*-glycolic acid) are the most commonly used biodegradable polymers in injectable drug delivery formulations (e.g., Lupron Depot®, Zoladex Depot®).

Hydrogels used for injectable cell delivery vehicles include alginates, poly(aldehyde guluronate) and adipic acid dihydrazide.[104] The latter two polymers have a wide range of mechanical stiffness and controllable degradation rate. Alginate gels exhibit a limited range of mechanical properties and have uncontrollable disintegration times.

Table 6. Examples of Excipients Used To Sustain and/or Control the Released of Drugs in Injectable Drug Delivery Systems[102]

Synthetic polymers for nanosphere and microsphere products
Polyesters
Polylactide
Polyglycolide
Poly(lactide-*co*-glycolide)
Polycaprolactones
Polyanhydrides
Poly(biscaroxyphenoxy) propane-cosebacic acid
Polyphosphazenes
Poly(dichlorophosphazene)
Polymer blends
Polycaprolactone + poly(hydroxybutyric acid + poly(lactide-*co*-glycolide)
Nano/Micropheres from natural polymers
Albumin
Collagen
Fibrinogen
Gelatin
Alginate
Cellulose
Chitan and Chitosan
Dextran
Hyaluronate
Starch
In situ gelling systems
Polyethylene oxide–polypropylene oxide (PEO–PPO) (Pluronic® F 127)
N-isoproplylacrylamide (NIPA)
Glycerol monooleate (GMO)
Atrigel® (PLA, PLGA, and copolymers of DL-lactide with caprolactone)
Sucrose acetate isobutyrate (SAIB)
Acrylate-terminated polymers
Acrylate-terminated PEG–PLA
Liposomes
Hydrogenated soy phosphatidylcholine
Distearoylphosphatidylclycerol
Dioleoyl phosphatidylethandamine (DOPE)
Dioctadecylamidoglycylspermine (DOGS)
Other general polymers
Polyvinyl alcohol
Polyamides
Poly(ethylene oxide)
Poly(ethylene glycol)

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ENDOCRINE RESEARCH COMMUNICATIONS, 8(2), 83-95 (1981)

PHENOL, A POTENT STIMULATOR OF ADENYLATE CYCLASE IN HUMAN THYROID MEMBRANES

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ABSTRACT

Among several commercial hCG preparations tested for their ability to stimulate adenylate cyclase in a human thyroid particulate fraction, only a pharmaceutical preparation (APL®) was active. Activity of this preparation was lost during dialysis, but could be restored fully by the addition of phenol and partially by the addition of benzyl alcohol, the two additives present in APL®. Phenol itself (0.040 - 2.0 mg/ml) induced a potent, dose-dependent stimulation of adenylate cyclase activity in a human thyroid particulate fraction and was also active in plasma membranes from rat liver and kidney cortex. Phenol exerted a biphasic effect on [^{125}I]-bTSH binding to human thyroid membranes. Concentrations between 0.33 and 3.3 mg/ml were stimulatory to binding, while higher concentrations were inhibitory.

Introduction

In previous studies, in an attempt to ascertain whether or not hCG has significant stimulating activity for the human thyroid, we have examined

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the effect of several commercial preparations of crude hCG[2], as well as that of a highly purified hCG distributed by the National Institutes of Health, on adenylate cyclase activity in human thyroid particulate fractions (1). Though all other preparations tested were without activity, a pharmaceutical preparation of hCG (APL®) did produce a pronounced stimulation of adenylate cyclase activity (1).

We now present evidence indicating that this activity of APL® in human thyroid membranes is largely due to the phenol which is added to this preparation as a preservative; that phenol itself is a potent stimulator of adenylate cyclase activity in human thyroid tissue; and that it is active in other tissues, as well.

Materials and Methods

Purified bTSH (approximately 30 IU/mg) employed for radioiodination was a gift from Dr. John G. Pierce, University of California, Los Angeles. Some preparations of crude hCG (2,400 IU/mg) were a gift from Dr. Morris L. Givner of Ayerst Laboratories, Montreal, Canada. Other preparations of crude hCG were purchased from Ayerst, Sigma, and Calbiochem Laboratories. Also purchased from Ayerst Laboratories was a pharmaceutical preparation of hCG (APL®) said to contain in each vial 10,000 USP units of hCG, 200 mg benzyl alcohol, 180 mg lactose and no more than 20 mg phenol.

2. Abbreviations used are: hCG, human chorionic gonadotropin; bTSH, bovine thyrotropin; cyclic AMP, adenosine 3'5'-monophosphate; ITP, inosine 5'-triphosphate; BSA, bovine serum albumin; bPTH, bovine parathyroid hormone.

Bovine PTH (1-84 residues, 3,000 MRC units/mg) was kindly provided by Dr. Gino Segre of Massachusetts General Hospital, Boston, and porcine glucagon by Dr. Mary Root of Eli Lilly Corporation, Indianapolis, Indiana. Phenol and benzyl alcohol were purchased from Fisher Scientific Company and lactose from Sigma Chemical Company.

Human thyroid membranes employed for binding studies were prepared by methods described previously (1), using frozen thyroid glands that had been removed at surgery from patients with Graves' disease. The particulate fraction employed in adenylate cyclase assays was obtained from glands collected and processed immediately after surgery according to the technique of Orgiazzi et al. (2).

Plasma membranes from rat liver were prepared by differential centrifugation and were purified by passage on a discontinuous sucrose gradient (3). Membranes from rat kidney cortex were prepared by procedures described by Zull and associates (4).

Protein content of the plasma membrane preparations was determined by the method of Lowry et al., using BSA as a standard (5).

Adenylate cyclase activity in the membrane preparations was evaluated by measuring the increase in cyclic AMP concentrations of the medium during 1 h of incubation with the test agents. The incubation medium contained 3 mM Tris ATP, 6 mM $MgCl_2$, 10 mM phosphocreatine, 0.3 mg/ml creatine phosphokinase, and 1 mM methyl isobutylxanthine (MIX), a potent phosphodiesterase inhibitor, in a total volume of 0.25 ml of 25 mM Tris-Cl, pH 7.6, containing 0.1% BSA. In experiments designed to study the effect of TSH and other test agents on adenylate cyclase in human thyroid membranes, the incubation medium also contained 0.05 mM ITP. Incuba-

tions were carried out at 37 C, and reactions initiated by the addition of membrane (50 ug protein) to the medium. Basal activity was determined in vessels that contained all the reagents except the hormones or test agents. In control experiments, incubations were carried out in media that contained all the reagents and test agents, but lacked membranes. Reactions were terminated by heating the specimens in a boiling water bath for five minutes. Cyclic AMP concentration was measured by radioimmunoassay in an aliquot of the medium.

Binding of $[^{125}I]$-bTSH to human thyroid membranes was studied in 20 mM Tris-Cl buffer, pH 7.45, containing 0.5% BSA; incubations were conducted for 2.5 h at 4 C, as described previously (1,6). Non-saturable binding of bTSH was measured in the presence of an excess (3.3 IU/ml; 3 x 10^{-6} M)[3] of unlabeled bTSH (Thytropar®). Gonadotropic activity of hCG preparations was assessed by a radio-receptor assay, employing a particulate fraction from rat testis, as described earlier (6).

Results

Responsiveness of adenylate cyclase in the thyroid membrane preparation to physiological stimulation was demonstrated by experiments in which bTSH enhanced enzyme activity in a dose-dependent manner over a concentration range between 0.4 and 20 mIU/ml (3.6 x 10^{-10}-1.8 x 10^{-8}M).

3. The molecular weight and biological activity of bTSH have been assumed to be 28,000 and 40 IU/mg, respectively. The molecular weight value used for glucagon was 3,550.

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In the same experiments, in confirmation of our earlier results (1), APL® (1000-3000 IU/ml; $2 - 6 \times 10^{-6}$M) also produced a concentration-dependent stimulation of adenylate cyclase activity. In contrast, no stimulation of adenylate cyclase was produced by dialyzed APL® in concentrations that contained amounts of hCG equal to those contained in undialyzed APL®, as judged from the testis radioreceptor assay.

Experiments were then conducted to determine whether the stimulatory activity of APL® that was lost during dialysis could be restored by the addition of one or more of the several additives present in the original, undialyzed preparation. When phenol was added to dialyzed APL® in a quantity approximately equivalent to that present in the stimulatory concentration of undialyzed APL®, the cyclase-stimulating activity of the reconstituted mixture was at least as great as that of undialyzed APL® itself (Figure 1). Moreover, phenol itself, in the absence of APL®, produced a striking biphasic response of adenylate cyclase in the human thyroid membrane preparation (Figure 2). Concentrations of phenol between 0.04 and 2.0 mg/ml ($0.4 \times 10^{-3} - 2 \times 10^{-2}$ M) produced a marked concentration-dependent stimulation; this was followed by a sharp decline when the phenol concentration was 3.0 mg/ml (3×10^{-2}M). Benzyl alcohol (2 mg/ml; 1.8×10^{-2}M) produced an approximately 80% increase in cAMP concentration over the basal level when studied alone and a 70% increase when added to dialyzed APL® (1,000 IU/ml). Higher concentrations either caused no further increase (4 mg/ml; 3.6×10^{-2} M) or were inhibitory (8 mg/ml; 7.2×10^{-2}M); lactose (18 or 36 mg/ml; 5×10^{-2} or 1×10^{-1} M) had no effect, either when tested alone or when added to dialyzed APL® (1,000 IU and 2,000 IU/ml) (data not shown).

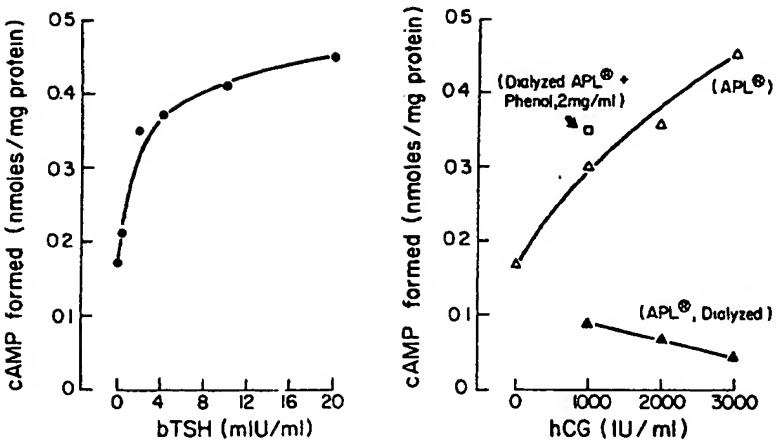
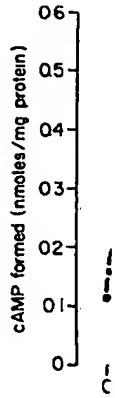


FIGURE 1

Effect of bTSH and APL® on adenylate cyclase activity in the human thyroid particulate fraction. Left: Human thyroid particulate fraction was incubated with increasing concentrations of bTSH (●—●). Right: The thyroid particulate fraction was incubated with APL® (△—△), dialyzed APL® (▲—▲) or dialyzed APL® to which phenol was added (□). Results shown here, and in subsequent figures, are means of duplicate or triplicate determinations.

The effect of phenol on the binding of $[^{125}I]$-bTSH to human thyroid membranes was also studied. At concentrations between 0.33 and 3.3 mg/ml (3.3×10^{-3} - 3.3×10^{-2} M), it produced a progressive enhancement of the binding (Figure 3). The maximum increase was noted in the presence of 3.3 mg/ml (3.3×10^{-2}M) of phenol, at which concentration the binding of $[^{125}I]$-bTSH was 144% of the control value (11% of added $[^{125}I]$-bTSH).

PHENOL



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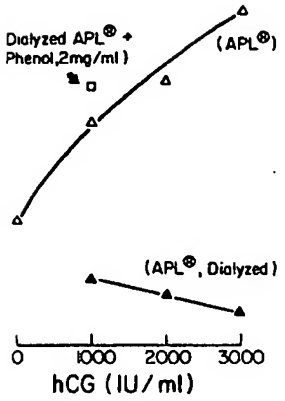

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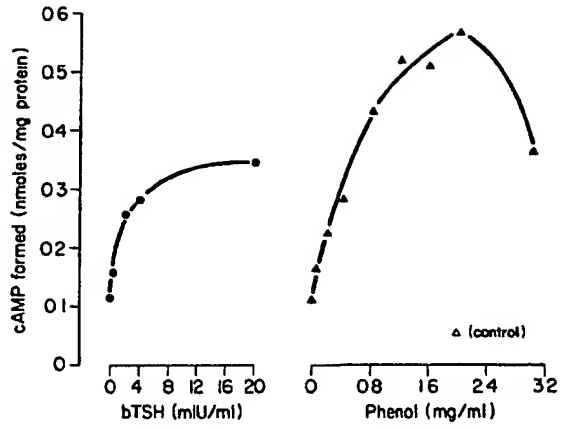


FIGURE 2

Activation of adenylate cyclase in the human thyroid particulate fraction by bTSH and phenol. The thyroid particulate fraction was incubated either with increasing concentrations of bTSH (●—●) (left), or phenol (▲—▲)(right). In a control experiment, the indicated concentration of phenol was incubated with the assay medium in the absence of the thyroid particulate fraction (▲).

Higher concentrations of phenol were inhibitory, since the binding of [^{125}I]-bTSH was reduced to 87% of the initial value in the presence of 5mg/ml (5 x 10^{-2} M) of phenol.

The tissue specificity of the stimulatory effect of phenol on adenylate cyclase activity was explored by studying its effect on plasma membranes of rat liver and kidney. At a concentration range of 0.2 - 2 mg/ml (2 x 10^{-3} - 2

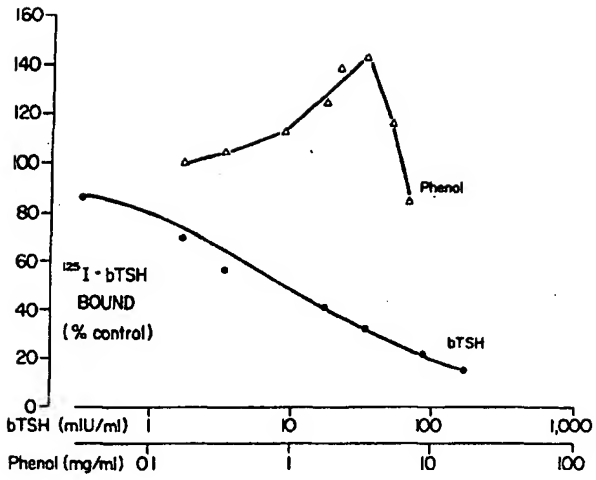
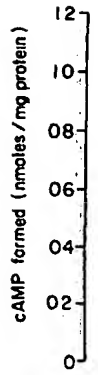


FIGURE 3

Effect of phenol on the binding of [^{125}I]-bTSH to human thyroid membranes. [^{125}I]-bTSH (approximately 8,000 cpm) was incubated with human thyroid membranes (66 ug membrane protein/ml) in the presence of increasing concentrations of either bTSH (●—●) or phenol (△—△). The binding was assessed following incubation at 4 C for 2.5 h. Results shown are typical of those obtained in 3 separate experiments in which triplicate vessels were studied for each point. In these experiments, binding of tracer concentrations of [^{125}I]-bTSH ranged between 11% and 18%.

x 10^{-2} M), phenol was stimulatory to adenylate cyclase in plasma membranes from rat kidney cortex (Figure 4). As in the human thyroid particulate fraction, the effect was biphasic, high concentrations (3 mg/ml; 3 x 10^{-2} M) of phenol being sharply inhibitory. In rat liver membranes, both glucagon (20 - 200 ng/ml; 6 x 10^{-9} - 6 x 10^{-8} M) and phenol (0.2 -1.6 mg/ml;



Activation of a
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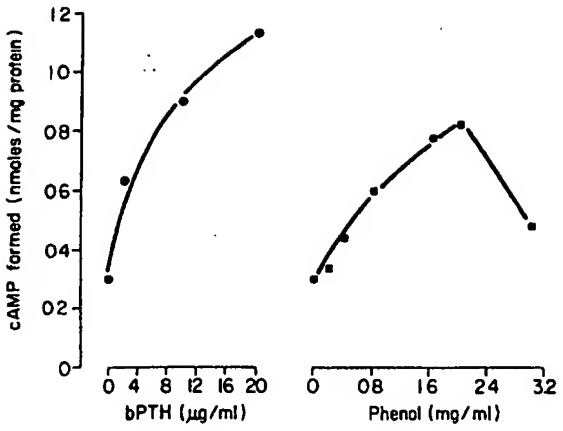

FIGURE 4

Activation of adenylate cyclase in rat kidney cortex plasma membranes by bPTH and phenol. Rat kidney cortex plasma membranes were incubated either with increasing concentrations of bPTH (●—●) (left) or phenol (●—●) (right).

2×10^{-3} - 1.6×10^{-2} M) were stimulatory to adenylate cyclase (Figure 5). However, the maximum response to phenol was much less than that to glucagon. Furthermore, the magnitude of the maximum stimulatory effect of phenol in liver membranes was much lower than had been noted either in membranes of kidney cortex or thyroid particulate fraction (Figures 2 and 4).

Discussion

APL® is a pharmaceutical preparation of crude hCG that we have found capable of stimulating adenylate cyclase activity in human thyroid membranes (1). The present data provide, however, several lines of

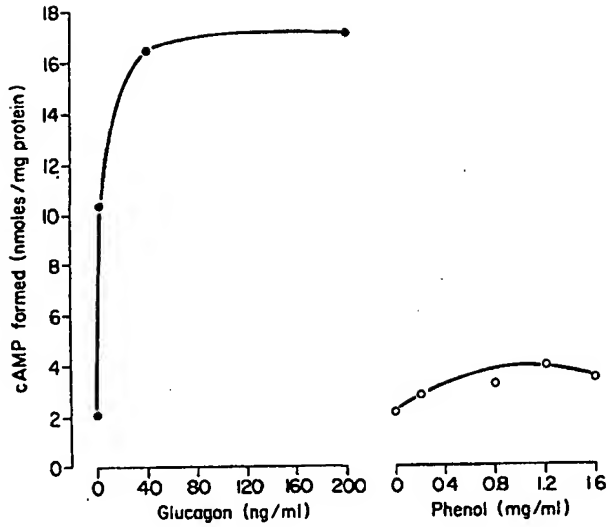

FIGURE 5

Effect of glucagon and phenol on adenylate cyclase activity in rat liver plasma membranes. Rat liver plasma membranes were incubated with increasing concentrations of either glucagon (●—●) (left) or phenol (○—○) (right).

evidence that it is the added preservatives, phenol and benzyl alcohol, rather than hCG or other factors isolated from urine with it, that are responsible for this activity. First, several other preparations of crude hCG that contained no preservative, including some provided by the manufacturer of APL®, displayed no adenylate cyclase-stimulating activity. Second, dialysis caused APL® to lose its activity, but this could be restored fully by the addition of the original concentration of phenol, and partly by the addition of benzyl alcohol. Finally, phenol itself was shown to be a potent,

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and benzyl alcohol a somewhat less potent, direct stimulator of adenylate cyclase activity in thyroid particulate fractions.

Earlier studies by others have revealed that a number of short-chain aliphatic alcohols are capable of activating glucagon-responsive adenylate cyclase in particulate fractions or whole homogenates of rat liver (7). Similarly, certain aliphatic and aromatic alcohols, especially ethanol, have been shown by Atkinson et al. to cause a significant increase in cyclic AMP concentration in human peripheral blood lymphocytes, platelets, granulocytes and lymphocyte membranes (8). Ethanol has also been shown to be an effective activator of adenylate cyclase activity in plasma membranes from bovine thyroid, rat liver and kidney membranes, as well as in homogenates of placenta (9,10).

In common with the effect of ethanol, the adenylate cyclase-stimulating effect of phenol, a chemical belonging to the family of aromatic alcohols, was not specific to thyroid since, in our studies, a variable degree of stimulation was also observed in membranes from rat kidney and liver. Unlike the effect of ethanol, which is very similar in all three tissues, that of phenol was very vigorous in thyroid and kidney, but only weak in liver membranes (9).

The mechanism by which phenol activates adenylate cyclase activity is not clear. There was no direct correlation between the concentrations of phenol (330 ug/ml - 3.3 mg/ml) that enhanced $[^{125}I]$-bTSH binding to human thyroid membranes and the concentrations that activated adenylate cyclase activity therein (40 ug/ml - 3 mg/ml). It seems plausible that both the increased binding of $[^{125}I]$-bTSH and the enhancement of adenylate cyclase activity are mediated through a nonspecific effect of phenol on membrane

configuration, as has been postulated in the case of the stimulatory effect of ethanol on bovine thyroid adenylate cyclase (9).

In conclusion, the present studies have demonstrated that phenol is a potent stimulator of adenylate cyclase activity in human thyroid tissue. This activity can account for the stimulation of adenylate cyclase produced by certain pharmaceutical preparations of hCG that contain phenol as an additive. Furthermore, since chemical additives such as phenol and benzyl alcohol may be present in other pharmaceutical preparations employed in in vitro studies, our results emphasize the need for caution in the interpretation of such studies when adenylate cyclase activity is used as an index of response.

Acknowledgements

These studies were supported in part by Grant No. AM-18416 from the National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland, and a grant from the William F. Milton Foundation of Harvard University, Boston, Massachusetts.

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A.P.L.® Injection 5 000 IU
A.P.L.® Injection 10 000 IU

CAL

SCHEDULING STATUS:
S4

PROPRIETARY NAME
(and dosage form):

A.P.L.® Injection 5 000 IU
A.P.L.® Injection 10 000 IU
plus Sterile Diluent for A.P.L.®Injection

COMPOSITION:
A.P.L. (human chorionic gonadotropin (HCG)) is biologically standardised and the potency is declared in terms of the second International Standard for Chorionic Gonadotropin. Each unit represents the specific gonadotropic activity of 0,001279 mg of the standard preparation held by the National Institute for Medical Research (England) on behalf of the World Health Organisation.

When reconstituted with 10 mL of accompanying sterile diluent, the resulting solutions also contain 2,0% Benzyl alcohol as a pr preservative, not more than 0,2% phenol, and the following concentration of (lactose: APL 5 000: 0,9%; APL 10 000:1,8%.

PHARMACOLOGICAL CLASSIFICATION:
Category A, 21.10 Trophic hormones.

PHARMACOLOGICAL ACTION:
Chorionic gonadotropin is a hormone of human pregnancy; it is secreted by the syncytiotrophoblast of foetal placenta as early as 7 days after ovulation, and it is absorbed into the blood in sufficient quantity to sustain luteal function and forestall the next menstrual period. The secretion of LH therefore remains suppressed because of the rising concentrations of oestrogen and progesterone (Lipsett and Ross, 1978).

Peak levels of serum HCG are reached between the eighth and twelfth weeks of gestation. Thereafter, the levels decline reaching a nadir in the second trimester where they remain until parturition.

The changes in the corpus luteum in early pregnancy reflect the intense luteotrophic stimulation provided by the LH-like action of chorionic gonadotropin.

In the pregnant woman HCG is placentotrophic, increasing the output of oestrogens and progestogens from the placenta. An adrenotrophic effect on the foetus has also been demonstrated.

In the male, A.P.L. (chorionic gonadotropin) is given in an attempt to stimulate the interstitial cells of the testes (cells of Leydig) to produce androgen. The response may be considered similar to the effect produced by the interstitial cell-stimulating hormone (ICSH) from the anterior lobe of the pituitary (anterior pituitary-like).

A.P.L. is likely to be of benefit in conditions directly related to insufficient secretion of androgen provided the interstitial cells of the testes are capable of stimulation.

A.P.L. (human chorionic gonadotropin) has no known effect on fat mobilization, appetite or sense of hunger or body fat distribution. HCG has not been demonstrated to be effective adjunctive therapy in the treatment of obesity.

INDICATIONS:
In the female:

1. Infertility - Ovulation Induction:

   A.P.L. is used in the induction of ovulation after the carefully monitored stimulation of follicular maturation with either human menopausal gonadotrophins (HMG) or clomiphene citrate.

In the male:

2. Cryptorchidism not due to anatomic obstruction. A.P.L. may also be used
   (a) as a diagnostic aid to determine the need for surgery;
   (b) pre-operatively, with a view to facilitating the procedure by increasing the size of the testes and the length of the cords.
   (c) Postoperatively as an aid in preventing retraction of the testes.
3. In selected cases of male hypogonadism secondary to pituitary failure (delayed adolescence, hypogonodotropic eunuchoidism).

CONTRA-INDICATIONS:
Precocious puberty, prostatic carcinoma or other androgen dependent neoplasia, prior allergic reaction to chorionic gonadotropin.

tion—(with preservatives: phenol 0.25% and thimerosal [mercury derivative] 0.005%). One vial containing 10 mL of Bacteriostatic Water for Injection, USP (with preservative: phenylmercuric nitrate 0.001%). One 1 mL vial of normal horse serum (diluted 1:10) as sensitivity testing material with preservatives: thimerosal (mercury derivative) 0.005% and phenol 0.35%. Not returnable.
Manufactured by: Wyeth Laboratories Inc.,
Marietta, PA 17547.

ANTIVENIN (Micrurus fulvius) ℞
[*an "te ven 'in*]
(equine origin)
North American Coral Snake Antivenin

COMPOSITION

Each combination package contains one vial of lyophilized Antivenin (Micrurus fulvius) with 0.25% phenol and 0.005% thimerosal (mercury derivative) as preservatives (before lyophilization); one vial of diluent containing 10 mL of Bacteriostatic Water for Injection, U.S.P., with phenylmercuric nitrate (1:100,000) as preservative.

HOW SUPPLIED

Combination packages as described (not returnable).
Manufactured by Wyeth Laboratories Inc.,
Marietta, PA 17547.
For prescribing information write to Professional Service, Wyeth-Ayerst Laboratories, P.O. Box 8299, Philadelphia, PA 19101, or contact your local Wyeth-Ayerst representative.

A.P.L.® ℞
(chorionic gonadotropin for injection, USP)
For Intramuscular Injection Only

Caution: Federal law prohibits dispensing without prescription.

DESCRIPTION

Human chorionic gonadotropin (HCG), a polypeptide hormone produced by the human placenta, is composed of an alpha and a beta subunit. The alpha subunit is essentially identical to the alpha subunits of the human pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as to the alpha subunit of human thyroid stimulating hormone (TSH). The beta subunits of these hormones differ in amino acid sequence.
A.P.L. (chorionic gonadotropin, USP) is a gonad-stimulating principle obtained from the urine of pregnant women. It is a sterile, amorphous powder prepared by cryodesiccation, and is freely soluble in water.
When reconstituted with the accompanying 10 mL of sterile diluent water, each SECULE® vial contains:
5,000 USP units of chorionic gonadotropin, 2.0% benzyl alcohol, 0.9% lactose, and not more than 0.2% phenol;
10,000 USP units of chorionic gonadotropin, 2.0% benzyl alcohol, 1.8% lactose, and not more than 0.2% phenol;
20,000 USP units of chorionic gonadotropin, 2.0% benzyl alcohol, 3.6% lactose, and not more than 0.2% phenol.
The pH is adjusted with sodium hydroxide or hydrochloric acid.
After reconstitution, store refrigerated and use within 30 days.
THIS PRODUCT IS FOR INTRAMUSCULAR INJECTION ONLY.

HOW SUPPLIED

A.P.L. (chorionic gonadotropin for injection, USP)
NDC 0046-0970-10 — Each package provides:
(1) One vial containing 5,000 USP units chorionic gonadotropin in dry form, and
(2) One 10 mL ampul sterile diluent.
NDC 0046-0971-10 — Each package provides:
(1) One vial containing 10,000 USP units chorionic gonadotropin in dry form, and
(2) One 10 mL ampul sterile diluent.
NDC 0046-0972-10 — Each package provides:
(1) One vial containing 20,000 USP units chorionic gonadotropin in dry form, and
(2) One 10 mL ampul sterile diluent.
The product is assayed in accord with USP method; USP potency units are defined in terms of the USP Chorionic Gonadotropin Reference Standard.
When reconstituted with 10 mL of accompanying sterile diluent, the resulting solutions also contain 2.0% benzyl alcohol, not more than 0.2% phenol, and the following concentrations of lactose: No. 970, 0.9%; No. 971, 1.8%; No. 972, 3.6%. The pH is adjusted with sodium hydroxide or hydrochloric acid.

DIRECTIONS FOR RECONSTITUTION

Withdraw sterile air from lyophilized vial and inject into sterile diluent vial. Remove 10 mL from diluent vial and add to lyophilized vial; agitate gently until powder is completely dissolved.
MAY BE STORED FOR 30 DAYS IN A REFRIGERATOR AFTER RECONSTITUTION.
For prescribing information write to Professional Service, Wyeth-Ayerst Laboratories, P.O. Box 8299, Philadelphia, PA 19101, or contact your local Wyeth-Ayerst representative.

ATIVAN® ℞
[*at 'i-van*]
(lorazepam)
Injection

DESCRIPTION

Ativan (lorazepam) Injection, a benzodiazepine with anti-anxiety and sedative effects, is intended for intramuscular or intravenous routes of administration. It has the chemical formula: 7-chloro-5-(o-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one. The molecular weight is 321.2, and the C.A.S. No. is [846-49-1].
Lorazepam is a nearly white powder almost insoluble in water. Each mL of sterile injection contains either 2.0 or 4.0 mg of lorazepam, 0.18 mL polyethylene glycol 400 in propylene glycol with 2.0% benzyl alcohol as preservative.

CLINICAL PHARMACOLOGY

Intravenous or intramuscular administration of the recommended dose of 2 mg to 4 mg of Ativan (lorazepam) Injection to adult patients is followed by dose-related effects of sedation (sleepiness or drowsiness), relief of preoperative anxiety, and lack of recall of events related to the day of surgery in the majority of patients. The clinical sedation (sleepiness or drowsiness) thus noted is such that the majority of patients are able to respond to simple instructions whether they give the appearance of being awake or asleep. The lack of recall is relative rather than absolute, as determined under conditions of careful patient questioning and testing, using props designed to enhance recall. The majority of patients under these reinforced conditions had difficulty recalling perioperative events or recognizing props from before surgery. The lack of recall and recognition was optimum within 2 hours following intramuscular administration and 15 to 20 minutes after intravenous injection.
The intended effects of the recommended adult dose of lorazepam injection usually last 6 to 8 hours. In rare instances and where patients received greater than the recommended dose, excessive sleepiness and prolonged lack of recall were noted. As with other benzodiazepines, unsteadiness, enhanced sensitivity to CNS-depressant effects of ethyl alcohol and other drugs were noted in isolated and rare cases for greater than 24 hours.
Studies in healthy adult volunteers reveal that intravenous lorazepam in doses up to 3.5 mg/70 kg does not alter sensitivity to the respiratory stimulating effect of carbon dioxide and does not enhance the respiratory depressant effects of doses of meperidine up to 100 mg/70 kg (also determined by carbon dioxide challenge) as long as patients remain sufficiently awake to undergo testing. Upper airway obstruction has been observed in rare instances where the patient received greater than the recommended dose and was excessively sleepy and difficult to arouse. (See "Warnings" and "Adverse Reactions".)
Clinically employed doses of lorazepam injectable do not greatly affect the circulatory system in the supine position or employing a 70-degree tilt test. Doses of 8 mg to 10 mg of intravenous lorazepam (2 to 2½ times the maximum recommended dosage) will produce loss of lid reflexes within 15 minutes.
Studies in six (6) healthy young adults who received lorazepam injection and no other drugs revealed that visual tracking (the ability to keep a moving line centered) was impaired for a mean of eight (8) hours following administration of 4 mg of intramuscular lorazepam and four (4) hours following administration of 2 mg intramuscularly with considerable subject variation. Similar findings were noted with pentobarbital, 150 and 75 mg. Although this study showed that both lorazepam and pentobarbital interfered with eye-hand coordination, the data are insufficient to predict when it would be safe to operate a motor vehicle or engage in a hazardous occupation or sport.
PHARMACOKINETICS
Injectable Ativan (lorazepam) is readily absorbed when given intramuscularly. Peak plasma concentrations occur approximately 60 to 90 minutes following administration and appear to be dose-related, e.g., a 2.0 mg dose provides a level of approximately 20 ng/mL and a 4.0 mg dose approximately 40 ng/mL in plasma. The mean half-life of lorazepam is about 16 hours when given intravenously or intramuscularly. Ativan (lorazepam) is rapidly conjugated at the 3-hydroxyl group into its major metabolite, lorazepam glucuronide, which is then excreted in the urine. Lorazepam glucuronide has no demonstrable CNS activity in animals. When 5 mg of intravenous lorazepam was administered to volunteers once a day for four consecutive days, a steady state of free lorazepam was achieved by the second day (approximately 52 ng/mL of plasma three hours after the first dose and approximately 62 ng/mL three hours after each subsequent dose, one day apart). At clinically relevant concentrations, lorazepam is bound 85% to plasma proteins.

INDICATIONS AND USAGE

Ativan (lorazepam) Injection is indicated in adult patients for preanesthetic medication, producing sedation (sleepiness or drowsiness), relief of anxiety, and a decreased ability to recall events related to the day of surgery. It is most useful in those patients who are anxious about their surgical procedure and who would prefer to have diminished recall of the events of the day of surgery (see "Precautions—INFORMATION FOR PATIENTS").

CONTRAINDICATIONS

Ativan (lorazepam) Injection is contraindicated in patients with a known sensitivity to benzodiazepines or its vehicle (polyethylene glycol, propylene glycol, and benzyl alcohol) and in patients with acute narrow-angle glaucoma. The use of Ativan (lorazepam) Injection intra-arterially is contraindicated because, as with other injectable benzodiazepines, inadvertent intra-arterial injection may produce arteriospasm resulting in gangrene which may require amputation (see "Warnings").

WARNINGS

PRIOR TO INTRAVENOUS USE, ATIVAN INJECTION MUST BE DILUTED WITH AN EQUAL AMOUNT OF COMPATIBLE DILUENT (SEE "DOSAGE AND ADMINISTRATION"). INTRAVENOUS INJECTION SHOULD BE MADE SLOWLY AND WITH REPEATED ASPIRATION. CARE SHOULD BE TAKEN TO DETERMINE THAT ANY INJECTION WILL NOT BE INTRA-ARTERIAL AND THAT PERIVASCULAR EXTRAVASATION WILL NOT TAKE PLACE.
PARTIAL AIRWAY OBSTRUCTION MAY OCCUR IN HEAVILY SEDATED PATIENTS. INTRAVENOUS LORAZEPAM, WHEN GIVEN ALONE IN GREATER THAN THE RECOMMENDED DOSE, OR AT THE RECOMMENDED DOSE AND ACCOMPANIED BY OTHER DRUGS USED DURING THE ADMINISTRATION OF ANESTHESIA, MAY PRODUCE HEAVY SEDATION; THEREFORE, EQUIPMENT NECESSARY TO MAINTAIN A PATENT AIRWAY AND TO SUPPORT RESPIRATION/VENTILATION SHOULD BE AVAILABLE.
There is no evidence to support the use of lorazepam injection in coma, shock, or acute alcohol intoxication at this time. Since the liver is the most likely site of conjugation of lorazepam and since excretion of conjugated lorazepam (glucuronide) is a renal function, this drug is not recommended for use in patients with hepatic and/or renal *failure*. This does not preclude use of the drug in patients with mild-to-moderate hepatic or renal disease. When injectable lorazepam is selected for use in patients with mild-to-moderate hepatic or renal disease, the lowest effective dose should be considered since drug effect may be prolonged. Experience with other benzodiazepines and limited experience with parenteral lorazepam has demonstrated that tolerance to alcoholic beverages and other central-nervous-system depressants is diminished when used concomitantly.
As is true of similar CNS-acting drugs, patients receiving injectable lorazepam should not operate machinery or engage in hazardous occupations or drive a motor vehicle for a period of 24 to 48 hours. Impairment of performance may persist for greater intervals because of extremes of age, concomitant use of other drugs, stress of surgery, or the general condition of the patient.
Clinical trials have shown that patients over the age of 50 years may have a more profound and prolonged sedation with intravenous lorazepam. Ordinarily, an initial dose of 2 mg may be adequate unless a greater degree of lack of recall is desired.
As with all central-nervous-system depressant drugs, care should be exercised in patients given injectable lorazepam as premature ambulation may result in injury from falling.
There is no added beneficial effect to the addition of scopolamine to injectable lorazepam, and their combined effect may result in an increased incidence of sedation, hallucination, and irrational behavior.
PREGNANCY
ATIVAN (LORAZEPAM) MAY CAUSE FETAL DAMAGE WHEN ADMINISTERED TO PREGNANT WOMEN. An increased risk of congenital malformations associated with the use of minor tranquilizers (chlordiazepoxide, diazepam, and meprobamate) during the first trimester of pregnancy has been suggested in several studies. In humans, blood levels obtained from umbilical cord blood indicate placental transfer of lorazepam and lorazepam glucuronide.
Ativan Injection should not be used during pregnancy. There are insufficient data regarding obstetrical safety of parenteral lorazepam, including use in cesarean section. Such use, therefore, is not recommended.

Continued on next page

PDR®

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EDITION

1997

PHYSICIANS' DESK REFERENCE®

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ISBN: 1-56363-201-2

Xylocaine Viscous permits complete filling of the stomach and the duodenal bulb while simplifying the passage of esophageal and gastric tubes.
ADMINISTRATION AND DOSAGE: Average dose—One tablespoonful, administered orally. See available literature for specific dosage instructions.
HOW SUPPLIED: Dispensed in 100 cc. and 450 cc. bottles.
LITERATURE AVAILABLE: Yes.

Aveeno Corporation

(Distributors E. Fougera & Co., Inc.)
250 W. 57TH ST.
NEW YORK 19, N.Y.

AVEENO® Colloidal Oatmeal
(for colloid baths)
COMPOSITION: The concentrated, specially-milled colloid fraction of oatmeal.
ACTION AND USES: For soothing and relief-promoting colloid baths in treating many itching and irritated skin conditions.
ADMINISTRATION AND DOSAGE: One cup to a tub of warm water once or twice daily, or as required. For infant baths, add 1 or 2 tablespoonfuls to the bathinette.
HOW SUPPLIED: 18 oz. and 4 lb. boxes.

AVEENO® Ointment
COMPOSITION: Aveeno Colloidal Oatmeal, zinc oxide and hexachlorophene in an emollient base.
ACTION AND USES: Aveeno Ointment, *the modernized Lassar's Paste,* is a soothing and protective ointment for use in a wide variety of skin problems. Because it contains colloidal oatmeal in place of starch, it has distinct advantages over Lassar's Paste, namely, greater absorbent and adhering capacity with increased soothing quality.
HOW SUPPLIED: In 1 oz. and 4 oz. tubes.

AVEENO® Soap Substitute
COMPOSITION: Aveeno Colloidal Oatmeal . . . in an emollient base.
ACTION AND USES: For cleansing irritated or sensitive skin. The possibility of irritation is markedly reduced because Aveeno Soap Substitute *does not* penetrate into the stratum corneum as do soap or "sudsing" soap-substitutes. Aveeno Soap Substitute merely "picks up" surface dirt by its excellent adsorptive quality. *Soothes as it cleanses.*
HOW SUPPLIED: In 3½ oz. tubes.

BUR-VEEN® Wet Dressing Powder
COMPOSITION: Aveeno Colloidal Oatmeal, aluminum sulfate, calcium acetate combines in solution to make modified Burow's solution with Colloidal Oatmeal).
ACTION AND USES: Wherever Burow's Solution is indicated—for weeping, inflamed, acute skin conditions. Supplies the therapeutic effect of Burow's Solution plus the soothing colloidal protection of Aveeno Colloidal Oatmeal.
ADMINISTRATION AND DOSAGE: One packet added to 1 pint of water makes the equivalent of a 1:20 Burow's Solution in a medium of soothing Aveeno Colloidal Oatmeal. Dilution may be varied as desired.
HOW SUPPLIED: Boxes of 6 and 100 packets.

PDR SECTIONS
PINK
ALPHABETICAL
INDEX
FIRMS AND PRODUCTS

Ayerst Laboratories

22 E. 40TH ST.
NEW YORK 16, N.Y.

"A.P.L."®
COMPOSITION: Brand of chorionic gonadotropin (human).
ACTION AND USES: In the male, "A.P.L." is given in an attempt to stimulate the interstitial cells of the testes (cells of Leydig) to produce androgen. The response to "A.P.L." may be considered similar to the effect produced by the interstitial cell-stimulating hormone (ICSH) of the anterior lobe of the pituitary. *For use* in cryptorchidism, delayed adolescence, dwarfism (pituitary), hypogonadotropic eunuchoidism, hypogonadism (after sexual maturity). *In the female,* "A.P.L." is administered in an attempt to maintain the functional integrity of the corpus luteum and to stimulate its secretion of progesterone. Response to "A.P.L." may be considered similar to the effect produced by the luteotropic hormone of the anterior pituitary gland. *For use* in abortion (habitual), amenorrhea, lobular hyperplasia, and sterility (functional).
ADMINISTRATION AND DOSAGE: For intramuscular injection only. In cryptorchidism, depending on the age of the patient, 4,000 I.U., three times weekly, for two to three weeks; or 1,000 I.U., three times weekly, for six to eight weeks. (Recommended dosages in all indications are given in the "A.P.L." package insert.)
CAUTION: In the male, evidence of sexual precociousness is an indication for the immediate withdrawal of treatment; however, therapy on a reduced dosage regimen may be resumed after regression of undue development in those cases in which such therapy is believed warranted.
HOW SUPPLIED: "Secule"® 20,000 I.U. w/10 cc. sterile diluent, pkg.; vials, 500 I.U./cc., 10 cc., and 1,000 I.U./cc., 10 cc. Rx only.
LITERATURE AVAILABLE: Yes.

"ANTABUSE"®
(ant' a buse)
Disulfiram
COMPOSITION: Brand of specially prepared and highly purified tetraethylthiuram disulfide.
ACTION AND USES: For use as an adjunct to the treatment of alcoholism. Creates an intolerance to alcohol and produces extreme physical discomfort when the patient under treatment ingests even small amounts of alcohol. "Antabuse" plus alcohol produces flushing, palpitations, dyspnea, hyperventilation, acceleration of pulse rate, fall in blood pressure, nausea, ultimately vomiting, and occasionally collapse. Drowsiness follows, with complete recovery after sleep.
ADMINISTRATION AND DOSAGE: "Antabuse" should be given only under close medical supervision. It should never be administered to a patient in a state of intoxication or without his full knowledge. The average initial dose is one tablet daily for two or three weeks, at which time an alcohol trial may be given. Maintenance dose, one-half tablet daily.
PRECAUTIONS: Supply of oxygen should be readily available in event of severe reaction following alcohol trial; other measures include administration of carbogen (95% oxygen and 5% carbon dioxide), intravenous vitamin C in massive doses (1 Gm.), ephedrine sulfate, or intravenous antihistamine.
CONTRAINDICATIONS: In patients with or suspected of having coronary or myocardial disease. In the presence of certain complicating diseases, special precautions must be observed. Detailed instructions are outlined in the literature.
HOW SUPPLIED: Tablets, 0.5 Gm. (scored), bottles of 50 and 1,000. Rx only.
LITERATURE AVAILABLE: Yes.

"BEMINAL"® FORTE WITH VITAMIN C
(bee' mi nal for' tay)
COMPOSITION: Each capsule contains:

Thiamine mononitrate (B_1)	25.0 mg.
Riboflavin (B_2)	12.5 mg.
Nicotinamide	75.0 mg.
Pyridoxine HCl (B_6)	3.0 mg.
Calc. pantothenate	10.0 mg.
Vitamin C (ascorbic acid)	150.0 mg.
Vitamin B_{12} with intrinsic factor concentrate	1/9 U.S.P. Unit

ACTION AND USES: For use pre- and postoperatively and whenever massive doses of vitamin B factors and vitamin C are indicated.
ADMINISTRATION AND DOSAGE: 1 to 3 capsules daily, or more, depending on the needs of the patient.
HOW SUPPLIED: Bottles of 100 and 1,000.
LITERATURE AVAILABLE: Yes.

"BEMOTINIC"® CAPSULES
(bee mo tin' ik)
COMPOSITION: Each capsule contains:

Ferrous sulfate exsic. (4½ gr.)	300 mg.
Vitamin B_{12} with intrinsic factor concentrate	½ U.S.P. Unit
Vitamin C (ascorbic acid)	100 mg.
Folic acid U.S.P.	1 mg.
Thiamine mononitrate (B_1)	10 mg.

ACTION AND USES: Provides all the elements known to be essential for hemoglobin formation and the development and maturation of erythrocytes, as well as other factors believed to be concerned with hemopoiesis. For use in the treatment of microcytic hypochromic anemia; maintenance and treatment of macrocytic hyperchromic anemias.
ADMINISTRATION AND DOSAGE: 1 or 2 capsules daily, or as indicated. Preferably taken before or after meals.
HOW SUPPLIED: Bottles of 100 and 1,000.
LITERATURE AVAILABLE: Yes.

"BEMOTINIC"® LIQUID
(bee' mo tin' ik)
COMPOSITION: Each teaspoonful (5 cc.) contains:

Ferric ammonium citrate	200.0 mg.
Vitamin B_{12}	4.0 mcg.
Extractive as obtained from of fresh gastric tissue	450.0 mg.
Folic acid U.S.P.	0.33 mg.
Thiamine HCl (B_1)	1.5 mg.
Riboflavin (B_2)	1.0 mg.
Pyridoxine HCl (B_6)	0.2 mg.

ACTION AND USES: A formulation of essential hemopoietic factors — orange-flavored, exceptionally palatable, nonalcoholic. Suggested for use in all anemias amenable to iron therapy; as a general blood-building tonic; as an adjunct to the treatment of macrocytic hyperchromic anemias with the exception of true Addisonian pernicious anemia.
ADMINISTRATION AND DOSAGE: For the treatment of microcytic nutritional anemias—*Adults:* 1 to 2 teaspoonfuls three times daily; *Children:* ½ to 1 teaspoonful three times daily. Preferably taken with meals. For other uses, as directed by physician.
HOW SUPPLIED: Bottles of 16 fluid-ounces and 1 gallon.
LITERATURE AVAILABLE: Yes.

"CLUSIVOL"® CAPSULES
(klus' i vol)
COMPOSITION: Two capsules contain:

Vitamin A	25,000 U.S.P. Units
Vitamin D	1,000 U.S.P. Units
Vitamin C	150.0 mg.
Vitamin B_1 mononitrate	10.0 mg.
Vitamin B_2	10.0 mg.
Vitamin B_6	1.0 mg.
Panthenol, equivalent to of calcium pantothenate	2.0 mg.
Vitamin B_{12} with intrinsic factor concentrate	½ U.S.P. Unit

Continued on next page

TWELFTH EDITION

PHYSICIANS' DESK REFERENCE

to

PHARMACEUTICAL SPECIALTIES and BIOLOGICALS

IN FIVE SECTIONS

An arbitrary page numbering plan to facilitate the compilation of this reference book.

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Editorial Consultant

PUBLISHED BY MEDICAL ECONOMICS, INC., ORADELL, N. J.

TION AND USES: Provides the soothing effect of Aveeno Colloidal Oatmeal (see ve) with the emolliency of 35% added Particularly effective in dry, chronic matoses such as senile pruritus, psoriasis winter itch. Also indicated in chickenmeasles and chronic atopic dermatitis.
MINISTRATION AND DOSAGE: adult bath, use 3 or 4 rounded tablefuls to tub of warm water. For infant b, add 2 or 3 level teaspoonfuls to bathte. Baths should be given once or twice y or as required.
W SUPPLIED: In 10 oz. cans.

EENO® OINTMENT

MPOSITION: Aveeno Colloidal Oatal, zinc oxide and hexachlorophene in an ollient base.
CTION AND USES: Aveeno Ointment a soothing and protective colloidal oatal ointment for a wide variety of skin oblems. Its distinct advantages are greatabsorbency, increased adhering capacity d superior soothing quality.
OW SUPPLIED: 1 oz. and 4 oz. tubes.

EN-VEEN® Wet Dressing Powder

MPOSITION: Aveeno Colloidal Oatal, aluminum sulfate, calcium acetate combines in solution to make modified urow's solution with Colloidal Oatmeal).
CTION AND USES: Wherever Burow's mition is indicated—for weeping, inflamed, in conditions. Supplies the therapeutic efect of Burow's solution *plus* the soothing rotection of Aveeno Colloidal Oatmeal.
DMINISTRATION AND DOSAGE: One packet added to 1 pint of water makes e equivalent of a 1:20 Burow's solution in medium of soothing Aveeno Colloidal Oatmeal. Dilution may be varied as desired.
OW SUPPLIED: 6 and 100 packets.

Ayerst Laboratories
685 THIRD AVE.
NEW YORK 17, N. Y.

ANTABUSE® ℞
Brand of Disulfiram

ACTION AND USES: For adjunctive treatment of alcoholism, to help the patient remain in a state of self-imposed sobriety. Patients on ANTABUSE (Disulfiram) therapy ingesting even small amounts of alcohol experience a highly unpleasant reaction, consisting of flushing, palpitations, dyspnea, hyperventilation, tachycardia, hypotension, nausea, vomiting, and occasionally collapse, followed by drowsiness with complete recovery after sleep. Severity of reaction varies with the individual and amount of ANTABUSE (Disulfiram) and alcohol ingested.
ADMINISTRATION AND DOSAGE: Before initiating therapy, abstinence from alcohol for at least 12 hours is necessary. Adequate blood levels are not attained before at least four days of administration. *Initial Dosage Schedule*—0.5 Gm. (1 tablet) daily, taken as an entire dose either a.m. or h.s., for two weeks. *Maintenance Regimen*—0.25 Gm. (¼ tablet) daily, ranging from 0.125 Gm. to 0.5 Gm. (¼ to 1 tablet) but not to exceed 0.5 Gm. (1 tablet) daily. Therapy may have to be continued on a daily basis for several months to years, depending on the individual.
SIDE EFFECTS: ANTABUSE (Disulfiram) has a low order of toxicity when used alone in the recommended dosage. Minor side effects, occasionally seen during the first few weeks of therapy include drowsiness, fatigability, impotence, headache, acneform eruptions, allergic dermatitis, or a metallic or garlic-like aftertaste. These symptoms often disappear spontaneously as therapy is continued or dosage reduced. Polyneuropathy and peripheral neuritis have been occasionally reported during prolonged therapy.
CONTRAINDICATIONS AND PRECAUTIONS: Contraindicated in severe myocardial disease or coronary occlusion; to be given with caution in the presence of diabetes mellitus, pregnancy, hypothyroidism, epilepsy, cerebral damage, chronic and acute nephritis. (While ANTABUSE [Disulfiram] *alone* is without known effect in these conditions, they may be aggravated by the hypotension associated with an alcohol-ANTABUSE [Disulfiram] reaction.) Should not be given in psychoses; and care should be taken in hepatic cirrhosis or insufficiency. Should not be given concomitantly with *paraldehyde*. When sedation is required or when addiction to *narcotics* or *sedatives* is superimposed on alcoholism, administration must be carefully controlled. ANTABUSE (Disulfiram) SHOULD NEVER BE ADMINISTERED TO A PATIENT WHEN HE IS IN A STATE OF ALCOHOL INTOXICATION NOR WITHOUT HIS FULL KNOWLEDGE. Patients must be fully advised of the symptoms of the alcohol-ANTABUSE (Disulfiram) reaction, the potential for which may persist for 10 to 14 days after cessation of therapy. Severe reactions should be treated with one or more of the following: oxygen by inhalation, carbogen (95% O_2 and 5% CO_2), massive intravenous doses of vitamin C (1.0 Gm.), ephedrine sulfate, or intravenous antihistamine. Patients should carry an appropriate *Identification Card* (available from Ayerst on request). A package insert (giving complete information on this product) is immediately available with each market package.
HOW SUPPLIED: No. 810—Each tablet contains 0.5 Gm. Disulfiram scored, in bottles of 50 and 1,000.

A.P.L.® ℞
Brand of chorionic gonadotropin

ACTION AND USES: In the male, A.P.L. (chorionic gonadotropin) is given in an attempt to stimulate the interstitial cells of the testes (cells of Leydig) to produce androgen. *For use* in cryptorchidism. (See package insert for other indications.) *In the female,* it is administered in the second phase of the cycle in an attempt to maintain the functional integrity of the corpus luteum and to stimulate its secretion of progesterone. (See package insert for other indications.)
ADMINISTRATION AND DOSAGE: For intramuscular injection only. *In cryptorchidism,* depending on the age of the patient, 4,000 I.U., three times weekly, for two to three weeks; or 1,000 I.U., three times weekly, for six to eight weeks. (Recommended dosages in all indications are given in the package insert.)
CAUTION: In the male, evidence of sexual precociousness is an indication for the immediate withdrawal of treatment; however, therapy on a reduced dosage regimen may be resumed after regression of undue development in those cases in which such therapy is believed warranted. Development of edema in males has been reported in a few instances where a high dosage regimen was being employed. This is usually regarded as a manifestation of salt and water retention attributable to androgen secretion resulting from stimulation of the testicular cells of Leydig. Dosage should be sharply reduced in such cases.
HOW SUPPLIED: A.P.L. (chorionic gonadotropin)—*In Solution*—No. 500—Each cc. contains 500 I.U. chorionic gonadotropin, in 10 cc. vials. No. 999—Each cc. contains 1,000 I.U. chorionic gonadotropin, in 10 cc. vials. These products also contain 0.8% sodium chloride and 0.5% phenol. The pH is adjusted to 7.2 with sodium hydroxide. A.P.L. (chorionic gonadotropin)—*In Dry Form*—No. 972—Each package provides: (1) One SECULE® containing 20,000 I.U. chorionic gonadotropin, and (2) one 10 cc. vial of sterile diluent.
When reconstituted with 10 cc. of accompanying sterile diluent, the resulting solution also contains 2.0% benzyl alcohol and 3.5% lactose. The pH is adjusted to 7.2 with sodium hydroxide.

AURALGAN® ℞

Each cc. contains:

Glycerin (dehydrated) (Contains not more than 0.6% moisture)	1.0 cc.
Antipyrine	54.0 mg.
Benzocaine	14.0 mg.

(Also contains 8-Hydroxyquinoline sulfate.)
AURALGAN is an effective dehydrating agent with hygroscopic and analgesic properties for treatment of "earache."
ACTION AND USES: Relief of pain and reduction of inflammation in congestive and serous stages of acute otitis media. Useful also in otitis externa ("swimmer's ear"), and for softening and removing cerumen.
AURALGAN contains Glycerin (dehydrated), 99.4%, the driest glycerin available for otic use. Providing maximum hygroscopic capacity because it is virtually free from moisture (not more than 0.6%), Glycerin (dehydrated) may be expected to withdraw excess moisture quickly, thus reducing swelling and inflammation simply and effectively. AURALGAN does not blanch or mask the tympanic membrane, and therefore does not distort the otoscopic picture.
ADMINISTRATION: Otitis media: With affected ear up, fill ear canal with AURALGAN. Permit the solution to run slowly along the wall of ear canal; this prevents entrapment of bubbles. Plug ear with cotton previously saturated with AURALGAN. Repeat every one to two hours (or three or four times a day). Use of a heat lamp or heating pad will also help in relieving pain. *Removal of cerumen:* Soft plug—Instill AURALGAN. After approximately 15 minutes, use moderately forceful syringing with warm water. Hard plug—Instill AURALGAN three times daily for two days to dehydrate ear canal and facilitate separation of plug. Then irrigate with warm water.
Note: Keep well closed. Keep dropper clean and dry.
HOW SUPPLIED: No. 1000—AURALGAN Otic Solution, package containing 15 cc. bottle with dropper.

BEMINAL® FORTE with VITAMIN C

Each capsule contains:

Thiamine mononitrate (Vit. B_1)	25.0 mg.
Riboflavin (Vit. B_2)	12.5 mg.
Niacinamide	50.0 mg.
Pyridoxine HCl (Vit. B_6)	3.0 mg.
Calcium pantothenate	10.0 mg.
Ascorbic acid (Vit. C)	250.0 mg.
Cyanocobalamin (Vit. B_{12})	2.5 mcg.

ACTION AND USES: Therapeutic B factors with ascorbic acid. For use pre- and postoperatively, and whenever massive doses of vitamin B factors and ascorbic acid are indicated.
ADMINISTRATION AND DOSAGE: Usual dosage: Adults—1 capsule daily, or as directed by physician.
HOW SUPPLIED: No. 817—BEMINAL Forte with Vitamin C Capsules, in bottles of 100 and 1,000.
[*Shown in Product Identification Section*]

CLUSIVETS®

Each tablet contains:

VITAMINS	
Vitamin A (as acetate)	10,000 U.S.P. Units
Ergocalciferol (Vit. D_2)	1,000 U.S.P. Units
Ascorbic acid (Vit. C)	50.0 mg.

continued on next page

NINETEENTH EDITION

PHYSICIANS' DESK REFERENCE

to

PHARMACEUTICAL SPECIALTIES and BIOLOGICALS

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IN SIX SECTIONS

An arbitrary page numbering plan is used to facilitate the compilation of this reference book.

PUBLISHED BY MEDICAL ECONOMICS, INC., ORADELL, N. J.

CBD

Ayerst—Cont.

BUSE, report that they are able to drink alcoholic beverages with impunity and without any symptomatology. All appearances to the contrary, such patients must be presumed to be disposing of their tablets in some manner without actually taking them. Until such patients have been observed reliably taking their daily ANTABUSE tablets (preferably crushed and well mixed with liquid), it cannot be concluded that ANTABUSE is ineffective.

DURATION OF THERAPY: The daily, uninterrupted administration of ANTABUSE must be continued until the patient is fully recovered socially and a basis for permanent self-control is established. Depending on the individual patient, maintenance therapy may be required for months or even years.

TRIAL WITH ALCOHOL: During early experience with ANTABUSE, it was thought advisable for each patient to have at least one supervised alcohol-drug reaction. More recently, the test reaction has been largely abandoned. Furthermore, such a test reaction should never be administered to a patient over 50 years of age. A clear, detailed and convincing description of the reaction is felt to be sufficient in most cases.

However, where a test reaction is deemed necessary, the suggested procedure is as follows:

After the first one to two weeks' therapy with 500 mg daily, a drink of 15 ml (½ oz) of 100 proof whiskey or equivalent is taken slowly. This test dose of alcoholic beverage may be repeated once only so that the total dose does not exceed 30 ml (1 oz) of whiskey. Once a reaction develops, no more alcohol should be consumed. Such tests should be carried out only when the patient is hospitalized, or comparable supervision and facilities, including oxygen, are available.

MANAGEMENT OF ANTABUSE-ALCOHOL REACTION: In severe reactions, whether caused by an excessive test dose or by the patient's unsupervised ingestion of alcohol, supportive measures to restore blood pressure and treat shock should be instituted. Other recommendations include: oxygen, carbogen (95 per cent oxygen and 5 per cent carbon dioxide), vitamin C intravenously in massive doses (1 g), and ephedrine sulfate. Antihistamines have also been used intravenously. Potassium levels should be monitored particularly in patients on digitalis since hypokalemia has been reported.

How Supplied: ANTABUSE—Each tablet (scored) contains 250 mg disulfiram, in bottles of 100 (NDC 0046-0809-81)—Each tablet (scored) contains 500 mg disulfiram, in bottles of 50 (NDC 0046-0810-50) and 1,000 (NDC 0046-0810-91).

[*Shown in Product Identification Section*]

A.P.L.®

Brand of chorionic gonadotropin for injection, U.S.P.
For Intramuscular Injection Only

Description: Human chorionic gonadotropin (HCG), a polypeptide hormone produced by the human placenta, is composed of an alpha and a beta subunit. The alpha subunit is essentially identical to the alpha subunits of the human pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as to the alpha subunit of human thyroid stimulating hormone (TSH). The beta subunits of these hormones differ in amino acid sequence.

A.P.L. (chorionic gonadotropin for injection, U.S.P.) is a gonad-stimulating principle obtained from the urine of pregnant women. It is an amorphous powder prepared by cryodesiccation, and is freely soluble in water.

Actions: The action of HCG is virtually identical to that of pituitary LH, although HCG appears to have a small degree of FSH activity as well. It stimulates production of gonadal steroid hormones by stimulating the interstitial cells (Leydig cells) of the testis to produce androgens and the corpus luteum of the ovary to produce progesterone. Androgen stimulation in the male leads to the development of secondary sex characteristics and may stimulate testicular descent when no anatomical impediment to descent is present. This descent is usually reversible when HCG is discontinued. During the normal menstrual cycle, LH participates with FSH in the development and maturation of the normal ovarian follicle, and the midcycle LH surge triggers ovulation. HCG can substitute for LH in this function.

During a normal pregnancy, HCG secreted by the placenta maintains the corpus luteum after LH secretion decreases, supporting continued secretion of estrogen and progesterone, and preventing menstruation. HCG HAS NO KNOWN EFFECT ON FAT MOBILIZATION, APPETITE OR SENSE OF HUNGER, OR BODY FAT DISTRIBUTION.

Indications: HCG HAS NOT BEEN DEMONSTRATED TO BE EFFECTIVE ADJUNCTIVE THERAPY IN THE TREATMENT OF OBESITY. THERE IS NO SUBSTANTIAL EVIDENCE THAT IT INCREASES WEIGHT LOSS BEYOND THAT RESULTING FROM CALORIC RESTRICTION, THAT IT CAUSES A MORE ATTRACTIVE OR "NORMAL" DISTRIBUTION OF FAT, OR THAT IT DECREASES THE HUNGER AND DISCOMFORT ASSOCIATED WITH CALORIE RESTRICTED DIETS.

1. Cryptorchidism not due to anatomic obstruction. In general, A.P.L. is thought to induce testicular descent in situations when descent would have occurred at puberty. A.P.L. thus may help to predict whether or not orchiopexy will be needed in the future. Although, in some cases, descent following A.P.L. administration is permanent, in most cases the response is temporary. Therapy is usually instituted between the ages of 4 and 9.
2. Selected cases of male hypogonadism secondary to pituitary failure.
3. Induction of ovulation and pregnancy in the anovulatory, infertile woman in whom the cause of anovulation is secondary and not due to ovarian failure, and who has been appropriately pretreated with human menotropins.

Contraindications: Precocious puberty, prostatic carcinoma or other androgen-dependent neoplasia, prior allergic reaction to chorionic gonadotropin.

Warnings: HCG should be used in conjunction with human menopausal gonadotropins only by physicians experienced with infertility problems who are familiar with the criteria for patient selection, contraindications, warnings, precautions, and adverse reactions described in the package insert for menotropins. The principal serious adverse reactions during this use are: (1) ovarian enlargement, ascites with or without pain, and/or pleural effusion, (2) rupture of ovarian cysts with resultant hemoperitoneum, (3) multiple births, and (4) arterial thromboembolism.

Precautions: Induction of androgen secretion by chorionic gonadotropin may induce precocious puberty in patients treated for cryptorchidism. If signs of precocious puberty occur, therapy should be discontinued.

Since androgens may cause fluid retention, chorionic gonadotropin should be used with caution in patients with epilepsy, migraine, asthma, cardiac or renal disease.

Adverse Reactions:
Headache
Irritability
Restlessness
Depression
Tiredness
Edema
Precocious puberty
Gynecomastia
Pain at site of injection

Dosage and Administration: There is a marked variance of opinion concerning the dosage regimens to be used. Therefore the regimen employed in any particular case will depend upon the indication for use, the age and weight of the patient, and the physician's preference. The following regimens have been advocated by various authorities.

Cryptorchidism: (Therapy is usually instituted between the ages of 4 and 9.)

(1) 4,000 U.S.P. Units three times weekly for three weeks.

(2) 5,000 U.S.P. Units every second day for four injections.

(3) 15 injections of 500 to 1,000 U.S.P. Units over a period of six weeks.

(4) 500 U.S.P. Units three times weekly for four to six weeks. If this course of treatment is not successful, another is begun one month later, giving 1,000 U.S.P. Units per injection.

Selected cases of male hypogonadism secondary to pituitary failure:

(1) 500 to 1,000 U.S.P. Units three times a week for three weeks, followed by the same dose twice a week for three weeks.

(2) 1,000 to 2,000 U.S.P. Units three times weekly.

(3) 4,000 U.S.P. Units three times weekly for six to nine months, following which the dosage may be reduced to 2,000 U.S.P. Units three times weekly for an additional three months.

Induction of ovulation and pregnancy in the anovulatory, infertile woman in whom the cause of anovulation is secondary:

5,000 to 10,000 U.S.P. Units one day following the last dose of menotropins.

How Supplied: A.P.L. (chorionic gonadotropin for injection, U.S.P.)

NDC 0046-0970-10 — Each package provides:
(1) One vial containing 5,000 U.S.P. Units chorionic gonadotropin in dry form, and
(2) One 10 ml ampul sterile diluent.

NDC 0046-0971-10 — Each package provides:
(1) One vial containing 10,000 U.S.P. Units chorionic gonadotropin in dry form, and
(2) One 10 ml ampul sterile diluent.

NDC 0046-0972-10 — Each package provides:
(1) One vial containing 20,000 U.S.P. Units chorionic gonadotropin in dry form, and
(2) One 10 ml ampul sterile diluent.

When reconstituted with 10 ml of accompanying sterile diluent, the resulting solutions also contain 2.0% benzyl alcohol, not more than 0.2% phenol, and the following concentrations of lactose: No. 970, 0.9%; No. 971, 1.8%; No. 972, 3.6%. The pH is adjusted with sodium hydroxide or hydrochloric acid.

MAY BE STORED FOR 90 DAYS IN A REFRIGERATOR AFTER RECONSTITUTION.

ATROMID-S®

Brand of clofibrate
Antilipidemic agent for reduction of elevated serum lipids

Actions: ATROMID-S is an antilipidemic agent. It acts to lower elevated serum lipids by reducing the very low density lipoprotein fraction (S_f20–400) rich in triglycerides. Serum cholesterol, especially the low density lipoprotein fraction (S_f0–20), is also decreased, particularly in those whose cholesterol levels are elevated at the outset.

The mechanism of action has not been established definitively. In man, clofibrate reduces cholesterol formation early in the biosynthetic chain. In addition, clofibrate has been shown to cause increased excretion of neutral sterols.

Animal studies suggest that clofibrate interrupts cholesterol biosynthesis prior to mevalonate formation.

PDR®

34 EDITION

1980

Physicians' Desk Reference®

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227 Abbildungen und 60 Tabellen

WVG

Wissenschaftliche Verlagsgesellschaft mbH Stuttgart 1985

	Phenol	o- m- p-Kresol	
Wirkung	bakteriostatisch bis bakterizid; nur schwach wirksam gegen Hefen und Pilze, virusid bei 2,5%	bakteriostatisch bis bakterizid	
Unverträglichkeiten	mit vielen organischen Stoffen kombinierbar; unverträglich auch z. B. mit Procain, Menthol, Natriumsalicylat u. vielen anderen Stoffen		
pH-Abhängigkeit	[illegible] unterhalb pH 8,5	s. Phenol	

	Chlorkresol	Hexachlorophen
Löslichkeit in Wasser (g/100 ml)	0,38 (20 °C)	sehr schwer löslich
Antimikrobielle Konzentrationen	0,1–0,2%	0,01–0,02%
Verwendung	[illegible]	für Seifen, Emulsionen, Lotionen
Wirkung	mikrobiostatisch bis mikrobizid, d. h. auch fungizid	mikrobiostatisch bis mikrobizid
Unverträglichkeiten	starke Sensibilisierung an [illegible]	Hautempfindlichkeit möglich
pH-Abhängigkeit	s. Phenol	s. Phenol

	PHB-Ester [illegible]	Propyl-	Thymol
Löslichkeit in Wasser (g/100 ml)	0,2 (20 °C) 2 (80 °C)	0,03 (20 °C) 0,3 (80 °C)	0,09 (20 °C)
Antimikrobielle Konzentrationen	0,2% In 0,09%iger Konzentration eines Gemisches aus 65% M.- u. 35% P.-Ester	0,04%	0,03%
Verwendung	für [illegible] und [illegible]		Oralia und kutane Arzneiformen
Wirkung	nur bakteriostatisch, keine [illegible] Pilze; Propylester schwach wirksam gegen Hefen		bakteriostatisch und fungistatisch
	0,2% PHB-Ester-Kombination + 0,5% Benzylalkohol im Gemisch sind gut wirksam gegen alle Mikroorganismen		
Unverträglichkeiten			
pH-Abhängigkeit			

	Benzylalkohol[21]	β-Phenyl-ethanol[21]	Chlorbutol Trichlor-tert-butylalkohol
Unverträglichkeiten			hohe Sorptionsneigung; im Neutralen und Alkalischen instabil
pH-Abhängigkeit			

Quecksilberverbindungen

	Kationische Hg-Verbindungen Phenylmercuri-nitrat, -borat, -acetat			Anionische Hg-Verbindung Thiomersal (Na-Salz der Äthylmercurithiosalicylsäure)
	-nitrat	-borat	-acetat	
Löslichkeit in Wasser (g/100 ml)	0,02 (20 °C)	0,03	0,17	50 (als Säure 0,02)
Antimikrobielle Konzentrationen	0,002–0,02%			0,006–0,02%
Verwendung	für [illegible] und [illegible] sowie orale und ophthalmologische Zwecke; Thiomersal auch für Injektionslösungen und Seren			
Wirkung[22]	In erster Linie bakteriostatisch bis bakterizid, aber auch fungistatisch bis fungizid. Hg-Ionen wirken nur mikrobiostatisch, während organische Hg-Verbindungen wegen ihrer Lipophilie auch mikrobizid wirken können. Hg-Verbindungen beeinträchtigen Zellfermente mit Mercapto-Gruppen, wodurch sämtliche Redoxprozesse unterbunden. Thiomersal wirkt nicht sporozid.			
Unverträglichkeiten	mit [illegible] Wirk- und Hilfsstoffen, mit Chlorid-, Bromid- und Jodid-Ionen; hohe Sorptionsneigung an Elastomere			instabil im neutralen und alkalischen Bereich; lichtempfindlich
pH-Abhängigkeit	wirken im [illegible] Bereich besser als im [illegible]			wirkt nur im Sauren unter pH 6 [illegible]

[22] Nach R. J. Fleury J. Pharm. Pharmac. 30, 128 (1978) sind plasmidhaltige Stämme von Escherichia coli, Pseudomonas aeruginosa und Staphylococcus aureus in der Lage, Hg-haltige Konservierungsmittel zu metallischem und damit flüchtigem Hg zu reduzieren, bis die MHK unterschritten ist und somit überlebende Keime sich wieder vermehren können.

Plasmide s. E. Reinhard: „Pharmazeutische Biologie", 2. Aufl., Wissenschaftl. Verlagsges., Stuttgart 1980.

Tabelle 18.8 Einsatzbereich einiger Konservierungsmittel. Die linke Gruppe kann nur im sauren, die rechte nur im alkalischen, die mittlere jedoch außer im neutralen auch im sauren und alkalischen pH-Bereich eingesetzt werden.

pH des zu konservierenden Arzneimittels bzw. des zu konservierenden Lebensmittels		
[illegible]	[illegible]	7–8
[illegible]	PHB-Ester	Chlorhexidin
Benzylalkohol	o-Phenylphenol	Phenylquecksilbersalze
	[illegible]	
Sorbinsäure		
Propionsäure	Thiomersal	
Dehydracetsäure		
p-Chlor-m-Kresol	Benzalkoniumchlorid	

18.3.3.1.3. Anwendung von Konservierungsmitteln

Der Gebrauch von Konservierungsmitteln ist in keinem Falle als Maßnahme aufzufassen, die vernachlässigte Produktionshygiene ausgleichen soll, sondern muß als Präventivmaßnahme gegen erstens unvermeidbare zufällige Kontaminationen während der Produktion und zum zweiten gegen unvermeidbare Kontaminationen, wie sie zwangsläufig bei der Entnahme der Arzneizubereitung aus einem Mehrdosenbehältnis entstehen, verstanden werden.

Bei der sehr wichtigen Konservierung von Augentropfen wird z. T. gefordert, daß das Konservierungsmittel nicht nur bakteriostatisch, sondern innerhalb von 1 Stunde bakterizid sein soll. (Wichtig für Augenkliniken mit häufiger Entnahme aus Mehrdosenbehältern!)

DAB 8 und Ph. Eur. machen zur Konservierung im Gegensatz zu anderen neuen Arzneibüchern keine Angaben; DAC enthält jedoch Monographien von Konservierungsstoffen ([illegible], Phenylquecksilberborat und -nitrat, Thiomersal) und schlägt auch Konzentrationen bestimmter Stoffe bei Arzneizubereitungen (z.B. Augentropfen) vor. Dabei liegen die von den Arzneibüchern vorgeschlagenen Konzentrationen meist im niedrigeren Bereich. USP XX fordert, daß eine ausreichend konservierte Augentropflösung oder Injektionslösung die Zahl der lebensfähigen Bakterien einer eingebrachten Kontamination innerhalb 14 Tagen auf 0,1% reduziert und daß während eines 28-Tage-Tests die überlebenden Keime sich nicht vermehren. Diese letzte Forderung gründet auf der Tatsache, daß die letzten überlebenden Keime in einer anfangs bakteriziden Lösung sich vermehren können unter Nutzung von Purinen und Pyrimidinen abgestorbener oder absterbender Zellen. Dieses Phänomen tritt jedoch nur bei den in niedriger Konzentration verwendeten hochaktiven Konservierungsmitteln auf; bei Konservierungsstoffen, die höher als 0,1% dosiert werden, ist es unbekannt.

18. Sterilised medicine forms

$$K = \frac{2.303}{t} \cdot \lg \frac{N_o}{N}$$

The curve 3 shown in Fig. 18.7 is produced, for example, when shown in graph form (in the case of a mathematically imprecise, but biologically correct reduction to the bacterial count 0).

The rise in the straight line produced from the bacterial count and time is dependent on the concentration of preservative and therefore also on the instant at which the bacterial count reduces to the value 0.

18.3.3.1.2 Preservative (see also 18.6.6)

All preservatives, owing to their chemical structure, have a certain amphiphilia, with the lipophilic character being predominant. The antimicrobial action is based on their cell toxicity. They are initially adsorbed on the microbe cell wall and then reach the interior of the cell by diffusion via the cytoplasm membrane. In this manner, they undergo reactions with the most varied cell components and lead to damage (for example phenols or surfactants) or act on cell protein (and on enzymes) in the form of lysis, coagulation, coacervation, structure change (for example heavy metal salts, dehydrating agents such as alcohols etc.).

The material used to preserve medicine forms can be divided into the following chemical classes:

1. Phenols
2. Carboxylic acids
3. Alcohols (aliphatic, aromatic, chlorinated)
4. Mercury compounds
5. Organic nitrogen compounds (quaternary ammonium compounds etc.)
6. Ethereal oils.

	p-Cl-*m*-cresol	Hexachlorophene
Solubility in water (g/100 ml)	0.38 (20°C)	very poor solubility
Antimicrobial concentrations	0.1-0.2%	0.01-0.02%
Use	for parenteral substances	for soaps, emulsions, lotions
Effect	microbiostatic to microbicidal, i.e. also fungicidal	microbiostatic to microbicidal
Incompatibilities	large tendency to sorption on elastomers	skin sensitisation possible
pH dependency	see phenol	see phenol

	Parabens		Thymol
	Methyl-	Propyl-	
Solubility in water (g/100 ml)	0.2 (20°C) 2 (80°C)	0.03 (20°C) 0.3 (80°C)	0.09 (20°C)
Antimicrobial concentrations	0.2%	0.04%	0.03%
	in 0.09% concentration of a mixture of 65% M.- and 35% P.-ester		
Use	For aqueous substances for peroral and external administration	Oral and cutaneous medicine forms	
Effect	Only bacteriostatic, no action against fungi; propyl ester poor action against yeasts 0.2% parabens combination + 0.54% benzyl alcohol mixed are effective against all micro-organisms	Bacteriostatic and fungistatic	
Incompatibilities			
pH dependency			

Aromatic and aliphatic alcohols

	Benzyl alcohol[20]	β-phenyl ethanol[21]	Chlorbutol Trichlor-*tert.*-butyl alcohol
Solubility in water (g/100 ml)	4 (17°C)	1.6-2	0.8 (20°C)
Antimicrobial concentrations	1-2%	0.7-1.5%	0.3-0.6%
Use	For aqueous and oil peroral, cutaneous, opthamological, nasal and parenteral preparations		For oily preparations for cutaneous, nasal, opthamalogical and parenteral application
Effect	Only poor against yeasts and fungi; only microbistatic, not microbicidal		Bacteriostatic to bactericidal
		Increase in effect by combination of 0.5% β-phenyl ethanol with 0.05% *p*-Cl-*m*-cresol	
Incompatabilities			High sorption tendency; unstable in neutral and alkaline range
pH dependency			

[20]The 4-chlor- and the 2,4 dichloro derivative of benzyl alcohol are used at 0.3 to 0.1%. They have a high sorption tendency.

[21]The *p*-Cl derivative (0.5% soluble) is used at 0.15 to 0.3% (bacteriostatic to bactericidal)

Nitrogen compounds

	Cetylpyridinium chloride	Benzalconium chloride	Chlorhexidine Bis-(*p*-Cl-phenyl-diguanido)-1,6-hexane-dihydrochloride or diacetate
Solubility in water (g/100 ml)	50	very soluble	0.8 as a base 1.9 as diacetate
Antimicrobial concentrations	0.0001-0.01%	0.0005-0.01%	0.001-0.01%
Use	For cutaneous, nasal and ophthalmological purposes, cetylpyridinium chloride also for oral and peroral purposes		Aqueous medicine forms for oral, peroral, cutaneous, nasal and particularly ophthalmological application
Effect	sporicidal action dubious; act by increasing the permeability of the cytoplasm membrane also on cell enzymes and cell proteins. The invert soaps have a wide action spectrum		Against bacteria and fungi; not sporicidal
Incompatabilities	with anion-active substances (for example Na-alginate); electrolytes, oxidising agents and some other materials, for example ephedrine, methyl cellulose etc. The high sorption tendency on interfaces of glass, metal and elastomers and migration into the phase interface of multi-phase preparations may prevent a preservative action		Incompatibilities such as in the case of invert soaps; high sorption tendency on elastomers
pH dependency	improved action in alkaline environment		

Not all microorganisms are equally sensitive to a preservative. Thus some materials have a more fungicidal action, others bactericidal or bacteriostatic.

Of the preservatives listed in the above tables, some materials are significant for the preservation of foodstuffs. However, some of the following are only common in food engineering: benzoic acid (and its Na salts), diethyl dicarbonate and dehydracetic acid (for mould fungi and yeasts, less effective against bacteria, for acid products with pH <

unavoidable contamination such as inevitably occurs when removing the medicinal preparation from a multi-compartment container.

In the very important preservation of eye drops, it is sometimes required that the preservative should be not only bacteriostatic but also bactericidal within 1 hour. (Important for eye clinics with frequent removal from multi-compartment containers!)

DAB 8 and Ph. Eur., in contrast to other new pharmacopoeias do not give any details on preservation; however, DAC contains monographs of preservatives (chlorhexadine acetate, phenylmercuric borate and nitrate, thiomersal) and also suggests concentrations of certain materials in medicine preparations (for example eye medicines). In this instance, the concentrations suggested by the pharmacopoeias are generally in the bactericidal range. USP XX requires that an adequately preserved eye drop solution should reduce the number of bacteria capable of survival of contamination which has been introduced to 0.1% within 14 days, and that the surviving germs no longer multiply during a 28 day test. This last requirement is based on the fact that the last surviving germs in an initially bactericidal solution can multiply using purines and pyrimidines of dead or dying cells. However, this phenomenon only occurs in the highly active preservatives used in low concentration; in the case of preservatives which have a higher than 0.1% dosage, it is unknown.

hypersensitivity to Dartal may occur rarely in patients who are known to be hypersensitive to drugs of a similar nature.

Since Dartal has not been studied extensively in pregnant women, it must be used with caution during pregnancy.

Pro-Banthine with Dartal contains thiopropazate hydrochloride, a tranquilizing drug. There is sufficient experimental evidence to conclude that chronic administration of antipsychotic drugs which increase prolactin secretion has the potential to induce mammary neoplasms in rodents under the appropriate conditions. There are recognized differences in the physiological role of prolactin between rodents and humans. Since there are, at present, no adequate epidemiological studies, the relevance to human mammary cancer risk from prolonged exposure to thiopropazate hydrochloride and other antipsychotic drugs is not known.

This product contains FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) sensitivity in the general population is low, it is frequently seen in patients who also have aspirin hypersensitivity.

Adverse Reactions: *Pro-Banthine:* Patients commonly complain of dryness of the mouth or blurring of vision, especially at high dosages. These complaints when reported are usually minor, disappear on continued treatment and necessitate no adjustment of dosage. Occasionally, however, they may be severe enough to require reduction in dosage, and then morning and/or noon doses may be reduced, while evening and night doses are maintained in order to provide maximal protection against high night secretions.

On a theoretical basis a curare-like action, although not reported, may occur with considerable overdosage. Under such circumstances loss of control of voluntary muscles will occur, the most important manifestation being the loss of control of the respiratory muscles. Such patients must receive prompt and continuing artificial respiration until the drug effect has been exhausted.

Dartal: The following adverse reactions have been associated with its use: pseudoparkinsonism (associated with high dosage, including muscular rigidity, fixed facies, tremor, ataxia, festinant gait and drooling), blurring of vision, xerostomia, hypotension, nasal congestion, leukopenia (rare), generalized erythema, constipation and allergic purpura.

Theoretically, since Dartal is a phenothiazine, side effects characteristic of this group of compounds may occur. Among these side effects, none of which have been known to occur with this drug, are the following: grand mal convulsions, altered cerebrospinal proteins, cerebral edema, atropine potentiation, potentiation of the effects of heat or of phosphorus insecticides, autonomic reactions, endocrine disturbances, reversed epinephrine effect, hyperpyrexia and pigmentary retinopathy.

Dosage and Administration: The average dose schedule of Pro-Banthine with Dartal for adults is one tablet three times daily. One or two additional tablets daily may be indicated in the individual patient.

How Supplied: Each compression-coated aqua-colored tablet has SEARLE debossed on one side and 641 on the other side, and contains 15 mg. of propantheline bromide and 5 mg. of thiopropazate hydrochloride; bottles of 100 and 500.

PRO-BANTHINE® with PHENOBARBITAL Tablets ℞

(Warning: may be habit forming)

Each Pro-Banthine with Phenobarbital tablet contains:

propantheline bromide	15 mg.
phenobarbital	15 mg.

(Warning: May be habit forming)

Propantheline bromide is (2-hydroxyethyl) diisopropylmethylammonium bromide xanthene-9-carboxylate.

For *Clinical Pharmacology* see Pro-Banthine Tablets.

Indications:

Based on a review of this drug by the National Academy of Sciences—National Research Council and/or other information, FDA has classified the indications as follows:

"Possibly" effective: as adjunctive therapy in the treatment of peptic ulcer and in the treatment of the irritable bowel syndrome (irritable colon, spastic colon, mucous colitis, acute enterocolitis, and functional gastrointestinal disorders).

Final classification of the less-than-effective indications requires further investigation.

For *Contraindications, Warnings, Precautions, Adverse Reactions,* and *Overdosage* see Pro-Banthine Tablets.

Dosage and Administration: The usual *adult dosage* of Pro-Banthine with Phenobarbital is one or two tablets three or four times daily.

For *Drug Interactions* see Pro-Banthine Tablets.

How Supplied: *For Oral Use:* Tablets: Pro-Banthine with Phenobarbital tablets containing 15 mg. of propantheline bromide and 15 mg. of phenobarbital are sugar coated, ivory colored, with SEARLE imprinted on one side and 631 on the other side; bottles of 100, 500, 1,000, and 2,500, and cartons containing 100 unit-dose, individually blister-sealed tablets.

[*Shown in Product Identification Section*]

The preceding prescribing information for Searle & Co. was current as of November 1, 1979.

Serono Laboratories, Inc.
11 BROOKS DRIVE
BRAINTREE, MA 02184

ASELLACRIN® ℞
(somatropin)
For Intramuscular Injection

Description: Asellacrin (somatropin) is a sterile, lyophilized, purified somatropic hormone extracted from the human pituitary gland.

The potency of Asellacrin (somatropin) is determined by bioassay in hypophysectomized rats and is designated in International Units (IU). Each 10 ml vial contains 10 IU somatropin and 40 mg of mannitol, and the pH is adjusted between 6 and 8 with hydrochloric acid and or sodium hydroxide.

Clinical Pharmacology: Asellacrin (somatropin) stimulates linear growth in patients with pituitary growth hormone deficiency.

Asellacrin (somatropin) is an anabolic agent that stimulates intracellular transport of amino acids and net retention of nitrogen, phosphorus and potassium. The intestinal absorption and urinary excretion of calcium are both increased. The serum concentration of phosphorus and alkaline phosphatase are increased. The synthesis of chondroitin sulfate and collagen, as well as the urinary excretion of hydroxyproline are also stimulated by Asellacrin (somatropin). Asellacrin (somatropin) stimulates intracellular lipolysis, increases the plasma concentration of free fatty acids and stimulates the oxidation of fatty acids. Intracellular glucose metabolism is inhibited, and there is decreased sensitivity to the action of insulin in response to the chronic administration of Asellacrin (somatropin).

Indications and Usage: Growth failure due to a deficiency of pituitary growth hormone is the only indication of Ascellacrin (somatropin) administration. Patients must have growth failure as evidenced by a growth rate that is subnormal for age in the absence of any cause other than growth hormone deficiency. Growth hormone deficiency must be documented by demonstrating failure of the serum growth hormone concentration to increase above 5–7 mg/ml in response to two standard stimuli. Standard stimuli which may be used are hypoglycemia, intravenous arginine, oral levodopa, intramuscular glucagon, or suitable modifications of these procedures.

Contraindications: Asellacrin (somatropin) is ineffective, and should not be used, in patients with closed epiphyses.

Asellacrin (somatropin) is contraindicated in the face of any progression of an underlying intracranial lesion.

Precautions: Asellacrin (somatropin) should be used only by physicians experienced in the diagnosis and management of patients with pituitary growth hormone deficiency.

Because of its diabetogenic actions, which include the induction of hyperglycemia and ketosis, Asellacrin (somatropin) should be used with caution in patients with diabetes mellitus or with a family history of diabetes mellitus. Regular urine testing for evidence of glycosuria should be carried out in all patients.

Subcutaneous administration of Asellacrin (somatropin) may lead to local lipoatrophy or lipodystrophy and may enhance the development of neutralizing antibodies. The injections must be intramuscular and the injection site should be rotated.

Bone age must be monitored annually during Asellacrin (somatropin) administration, especially in patients who are pubertal and/or receiving concomitant thyroid replacement therapy. Under these circumstances, epiphyseal maturation may progress rapidly to closure.

Concomitant glucocorticoid therapy may inhibit the response to Asellacrin (somatropin) and should not exceed 10–15 mg hydrocortisone equivalent/M^2 body surface area during the administration of Asellacrin (somatropin). Patients with growth hormone deficiency secondary to an intracranial lesion should be examined frequently for progression or recurrence of the underlying disease process.

Adverse Reactions: Antibodies to somatropin are formed in 30–40% of the patients who have received somatropin prepared by similar methods. In general, these antibodies are not neutralizing and do not interfere with the response to Asellacrin (somatropin) administration. Approximately 5% of treated patients developed neutralizing antibodies and failed to respond to somatropin. Therefore, testing for anti-somatropin antibodies should be carried out in any patient with well-documented growth hormone deficiency who fails to respond to therapy.

Dosage and Administration: Reconstitute each vial with 5 ml of Bacteriostatic Water for Injection (U.S.P.) only. Each vial of reconstituted Asellacrin (somatropin) provides 2 IU somatropin per ml, 10 IU per vial.

It is recommended that Asellacrin (somatropin) be given initially as 1 ml (2 IU) intramuscularly, three times a week, with a minimum of 48 hours between injections.

If at any time during continuous Asellacrin (somatropin) administraion the growth rate does not exceed 2.5 cm (1 in) in a 6-month period, the dose may be doubled for the next six months. This may be done with or without the

Continued on next page

Serono—Cont.

presence of antibodies to Asellacrin (somatropin). If there is still no satisfactory response, Asellacrin (somatropin) should be discontinued and the patient reinvestigated.

Treatment should be discontinued when the patient has reached a satisfactory adult height, when the epiphyses have fused, or when the patient ceases to respond to Asellacrin (somatropin) administration.

Storage: Unreconstituted vials of Asellacrin (somatropin) may be stored at room temperature (15°-30°C/59°-86°F).

Reconstituted vials must be refrigerated (2°-8°C/36°-46°F) and used within one month.

How Supplied: Each vial contains 10 IU somatropin in sterile, lyophillized form. There are four vials per carton.

Asellacrin (somatropin) will be supplied directly to physicians for patients who show clinical and laboratory evidence of growth hormone deficiency. Forms for providing supporting clinical and laboratory information to qualify patients for treatment may be obtained from MEDICAL AFFAIRS, Medical Research Dept., SERONO LABORATORIES, INC., Braintree, MA 02184.

Manufactured Expressly for:
SERONO LABORATORIES, INC.
Braintree, MA 02184 U.S.A.
by:
Ben Venue Laboratories, Inc., Bedford, OH 44146

PERGONAL® B
(menotropins)

Description: Pergonal® (menotropins) is a purified preparation of gonadotropins extracted from the urine of postmenopausal women. Each ampule of Pergonal® (menotropins) contains 75 I.U. of follicle-stimulating hormone (FSH) activity and 75 I.U. of luteinizing hormone (LH) activity.

Pergonal® (menotropins) is biologically standardized for FSH and LH (ICSH) gonadotropin activities in terms of the Second International Reference Preparation for Human Menopausal Gonadotropins established in September, 1964, by the Expert Committee on Biological Standards of the World Health Organization.

Actions: Pergonal® (menotropins) administered for nine to twelve days produces ovarian follicular growth in women who do not have primary ovarian failure. Treatment with Pergonal® (menotropins) in most instances results only in follicular growth and maturation. In order to effect ovulation, HCG (human chorionic gonadotropin) must be given following the administration of Pergonal® (menotropins) when clinical assessment of the patient indicates that sufficient follicular maturation has occurred.

Indications: Pergonal® (menotropins) and HCG given in a sequential manner are indicated for the induction of ovulation and pregnancy in the anovulatory infertile patient in whom the cause of anovulation is secondary and is not due to primary ovarian failure.

Pergonal® (menotropins) has proven effective in treating infertility in women with the following diagnoses: primary amenorrhea, secondary amenorrhea, secondary amenorrhea with galactorrhea, polycystic ovary syndrome, anovulatory cycles, irregular menses.

Selection of Patients:

1. Before treatment with Pergonal® (menotropins) is instituted, a thorough gynecologic and endocrinologic evaluation must be performed. This should include a hysterosalpingogram (to rule out uterine and tubal pathology) and documentation of anovulation by means of basal body temperature, serial vaginal smears, examination of cervical mucus, determination of urinary pregnanediol and endometrial biopsy.
2. Primary ovarian failure should be excluded by the determination of urinary gonadotropin.
3. Careful examination should be made to rule out the presence of an early pregnancy.
4. In patients with irregular bleeding, cancer of the endometrium and other possible organic causes of such bleeding should be ruled out before starting Pergonal® (menotropins) therapy.
5. Evaluation of the husband should be included in the workup.

Contraindications:

1. A high level of urinary gonadotropin indicating primary ovarian failure.
2. The presence of overt thyroid and adrenal dysfunction.
3. An organic intracranial lesion such as a pituitary tumor.
4. The presence of any cause of infertility other than anovulation, as stated in the indications.
5. In patients with abnormal bleeding of undetermined origin.
6. In patients with ovarian cysts or enlargement not due to polycystic ovary syndrome.
7. Pregnancy.

Warning: Pergonal® (menotropins) is a drug that should only be used by physicians who are thoroughly familiar with infertility problems. It is a potent gonadotropic substance capable of causing mild to severe adverse reactions. It must be used with a great deal of care.

Precautions:

1. Diagnosis Prior to Therapy.
   Careful attention should be given to diagnosis in candidates for Pergonal® (menotropins) therapy. (See sections headed "Indications" and "Selection of Patients").
2. Overstimulation of the Ovary During Pergonal® (menotropins) Therapy.
   In order to minimize the hazard associated with the occasional abnormal ovarian enlargement associated with Pergonal® (menotropins)-HCG therapy, the lowest dose consistent with expectation of good results should be used.
   Mild to moderate uncomplicated ovarian enlargement which may be accompanied by abdominal distension and/or abdominal pain occurs in approximately 20% of those treated with Pergonal® (menotropins) and HCG, and generally regresses without treatment within two or three weeks.
   The hyperstimulation syndrome characterized by sudden ovarian enlargement accompanied by ascites with or without pain and/or pleural effusion occurs in approximately 0.4% of patients when the recommended dose is administered. In studies performed, the overall incidence of the hyperstimulation syndrome was 1.3%.
   If hyperstimulation occurs, treatment should be stopped and the patient hospitalized. The hyperstimulation syndrome develops rapidly over a period of three to four days and generally occurs during the two week period immediately following treatment. The phenomenon of hemoconcentration associated with fluid loss in the abdominal cavity has been seen to occur and should be thoroughly assessed in the following manner: 1) fluid intake and output, 2) weight, 3) hematocrit, 4) serum and urinary electrolytes, and 5) urine specific gravity.
   These determinations are to be performed daily or more often if the need arises. Treatment is primarily symptomatic and would consist primarily of bed rest, fluid and electrolyte replacement and analgesics if needed. The ascitic fluid should never be removed because of the potential danger of injury to the ovary.
   Hemoperitoneum may occur from ruptured ovarian cysts. This is usually the result of pelvic examination. If this does occur, and if bleeding becomes such that surgery is required, the conservative approach with partial resection of the enlarged ovary or ovaries is generally adequate.
   Intercourse should be prohibited in those patients in whom significant ovarian enlargement occurs after ovulation because of the danger of hemoperitoneum resulting from ruptured ovarian cysts.
3. Arterial Thromboembolism
   Arterial thromboembolism following Pergonal® (menotropins) and HCG therapy has been reported in two patients, one of whom died (1).
4. Multiple Births
   Of the pregnancies following therapy with Pergonal® (menotropins) and HCG, 80% have resulted in single births and 20% in multiple births, most of which have been twins. Fifteen percent of the total pregnancies resulted in twins, of which 93% were viable (78 surviving infants from 43 sets of twins). Five percent of the total pregnancies have resulted in three or more conceptuses, of which only 20% were viable (nine surviving infants from three sets of triplets, four surviving infants from four sets of quadruplets, and no surviving infants from four sets of quintuplets). The patient and her husband should be advised of the frequency and potential hazards of multiple pregnancy before starting treatment.

Adverse Reactions:

1. Ovarian Enlargement
2. Hyperstimulation Syndrome
3. Hemoperitoneum
4. Arterial Thromboembolism
   (For details on these four adverse reactions, please refer to 'Precautions' above.)
5. Sensitivity to Pergonal® (menotropins)
   Three patients experienced febrile reactions after the administration of Pergonal® (menotropins). It is not clear whether or not these were pyrogenic responses or possibly allergic reactions.
6. Defects at Birth
   From 287 completed pregnancies following Pergonal® (menotropins)-HCG therapy, five incidents of birth defects have been reported. One infant had multiple congenital anomalies consisting of imperforate anus, aplasia of the sigmoid colon, third degree hypospadias, cecovesicle fistula, bifid scrotum, meningocele, bilateral internal tibial torsion, and right metatarsus adductus. Another infant was born with an imperforate anus and possible congenital heart lesions; another had a supernumerary digit; another was born with hypospadias and exstrophy of the bladder; and the fifth child had Down's syndrome. None of the investigators felt that these defects were drug-related.

Dosage and Administration:

FOR INTRAMUSCULAR ADMINISTRATION

1. Treatment for induction of ovulation
   Treatment with Pergonal® (menotropins) in most instances results only in follicular growth and maturation. In order to effect ovulation, HCG must be given following the administration of Pergonal® (menotropins) when clinical assessment of the patient indicates that sufficient follicular maturation has occurred. This is indirectly estimated by the estrogenic effect upon the target organs. These indices of estrogenic activity include:
   a) changes in the vaginal smear
   b) appearance and volume of the cervical mucus
   c) Spinnbarkeit, and
   d) fern ing of the cervical mucus.
   If available, the urinary excretion of estrogens is a more reliable index of follicular maturation.
   The clinical confirmation of ovulation, with the exception of pregnancy, is obtained by indirect indices of progesterone production.

for possible revisions

The indices most generally used a
lows:
a) a rise in basal body temperatur
b) change of the cervical mucu
"fern" pattern to a "cellular" pat
c) vaginal cytology characteristic
teal phase of the menstrual cycle
d) increase in urinary pregnanedic
e) menstruation following the shif
body temperature
Because of the subjectivity of th
tests for the determination of follic
uration and ovulation, it cannot be
phasized that the physician shoul
tests with which he is thoroughly
2. Dosage of Pergonal® (menotropin
The dose of Pergonal® (menotropi
duce maturation of the follicle mus
vidualized for each patient. It i
mended that the initial dose to an
should be 75 I.U. of FSH and 75 I.
(one ampule) per day, **ADMINISTE**
TRAMUSCULARLY for nine to tw
followed by HCG, 10,000 I.U., one
the last dose of Pergonal® (men
The hyperstimulation syndrome h
occurred with administration of 7
FSH and 75 I.U. of LH (one ampule
for up to twelve days. Administratio
gonal® (menotropins) should not e
days. The patient should be treat
indices of estrogenic activity, as
under Item 1 above, are equivale
greater than those of the normal in
If urinary estrogen determinations a
able, they may be useful as a guide
apy. If the total estrogen excretio
than 100 mcg/24 hours or the estri
tion is less than 50 mcg/24 hours
HCG administration, the hyperstir
syndrome is less likely to occur. If t
gen values are greater than this,
advisable to administer HCG bec
hyperstimulation syndrome is more
occur.
If the ovaries are abnormally enla
the last day of Pergonal® (men
therapy, HCG should not be adminis
this course of therapy; this will re
chances of development of the hyper
tion syndrome. If there is evidence
tion but no pregnancy, repeat this
regime for at least two more course
increasing the dose of of Pergonal®
tropins) to 150 I.U. of FSH and 150 I.
(two ampules) per day for nine to
days. As before, this dose should be
by 10,000 I.U. of HCG one day after
dose of Pergonal® (menotropins). 15
FSH and 150 I.U. of LH (two ampules
gonal® (menotropins) per day have
to be the most effective dose. If evi
ovulation is present, but pregnancy
ensue, repeat the same dose for tw
courses. Doses larger than this
recommended.
During treatment with both Per
(menotropins) and HCG and during
week post-treatment period, patient
be examined at least every other
signs of excessive ovarian stimulati
recommended that Pergonal® (men
administration be stopped if the ova
came abnormally enlarged or abo
pain occurs. Most of the ovarian hype
lation occurs after treatment has b
continued and reaches its maxim
about seven to ten days post-ovulati
tients should be followed for at le
weeks after HCG administration.
The couple should be encouraged
intercourse daily, beginning on the da
to the administration of HCG until ov
becomes apparent from the indices en
for the determination of progestatio
tivity. Care should be taken to insure
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PDR®
34
EDITION
1980

Physicians' Desk Reference®

Publisher • CHARLES E. BAKER, Jr.

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Serono Laboratories, Inc.
280 POND STREET
RANDOLPH, MA 02368

Serono Laboratories, Inc. will be pleased to answer inquiries about the following products:

ASELLACRIN® ℞
[a-sel'ah-crin]
(somatropin)

FOR SUBCUTANEOUS OR INTRAMUSCULAR INJECTION

Description: ASELLACRIN® (somatropin) is a sterile, lyophilized, purified somatropic hormone extracted from the human pituitary gland, the natural source of this hormone.

The potency of ASELLACRIN® is determined by in vitro radio-receptor assay.

The 5 ml vial contains 2 IU of somatropin and 20 mg of mannitol. The 10 ml vial contains 10 IU of somatropin and 40 mg of mannitol.

After reconstituting the 2 IU or 10 IU vial in 0.5 ml or 2.5 ml, respectively, for subcutaneous injection, each 0.5 ml of ASELLACRIN® contains 2 IU of somatropin and 20 mg or 8 mg, respectively, of mannitol, as well as other pituitary hormones as shown below. After reconstituting the 2 IU or 10 IU vial in 1 ml or 5 ml, respectively, for intramuscular injection, each 1.0 ml of ASELLACRIN® contains 2 IU of somatropin and 20 mg or 8 mg, respectively, of mannitol, as well as other pituitary hormones as shown below.

Follitropin (FSH)	less than 0.714 IU
Lutropin (LH)	less than or equal to 17.85 IU
Corticotropin (ACTH)	less than or equal to 0.014 IU
Thyrotropin (TSH)	less than 0.071 IU
Prolactin (PRL)	less than or equal to 2.86 IU

The pH is adjusted between 6 and 8 with sodium phosphate and sodium acid phosphate. The 2 IU vial contains 2.0 to 2.4 mg sodium phosphate and 0.3 to 0.4 mg sodium acid phosphate. The 10 IU vial contains 2.7 to 3.3 mg sodium phosphate and 0.4 to 0.5 mg sodium acid phosphate.

Clinical Pharmacology:

A. Skeletal Growth

ASELLACRIN® stimulates linear growth in patients with pituitary growth hormone deficiency. The measurable increase in growth (body length) after somatropin administration results from its effect on cartilaginous growth areas of the long bones. It is known that somatropin's effect is mediated by a sulfation factor, or somatomedin which permits the incorporation of sulfate into cartilage. Somatomedin is low in serum of the growth hormone deficient patients whose growth hormone deficiency is the result of hypopituitarism or hypophysectomy, whereas its presence can be demonstrated after somatropin therapy.

B. Cellular Growth

In addition to its effect on the skeleton, somatropin brings about an increase in the muscular and visceral mass. In muscle tissue the increase in mass is observed by a corresponding increase in number and dimension of muscular fiber cells.

C. Carbohydrate Metabolism

The diabetogenic effect of somatropin is well known in clinical medicine. Acromegalic patients often suffer from diabetes mellitus while hypopituitary children experience hypoglycemia. In healthy patients, very large doses of somatropin can interfere with glucose tolerance.

A simultaneous increase in the plasma insulin level is observed upon somatropin administration. The diabetogenic activity of somatropin is perhaps due to several concomitant factors:

a. Reduced transport of glucose into peripheral tissues.

b. Increased release of glucose from the liver

c. Reduced concentration of insulin at the muscular level.

d Reduced glycolysis resulting from the block of the enzyme triose phosphate dehydrogenase, mediated by non-esterified fatty acids.

D. Protein Metabolism

ASELLACRIN® is an anabolic agent that stimulates intracellular transport of amino acids and net retention of nitrogen, which can be quantitated by observing the decline in urinary nitrogen excretion and BUN. At the subcellular level, somatropin may stimulate the duplication of DNA, the synthesis of messenger ribonucleic acid (mRNA), the activation of cyclic AMP and the subsequent coupling of amino acids with their respective transfer RNA's. The increase of mRNA observed by some investigators may perhaps point to mRNA synthesis as the primary process in turn provoking protein synthesis.

E. Fat Metabolism

Somatropin stimulates intracellular lipolysis, increases the plasma concentration of free fatty acids and stimulates the oxidation of fatty acids. In the diabetic patient, somatropin has been shown to accentuate ketogenesis.

F. Connective Tissue Metabolism

Somatropin stimulates the synthesis of chondroitin sulfate and collagen as well as the urinary excretion of hydroxyproline.

G. Mineral Metabolism

Somatropin induces the net retention of phosphorus and potassium and to a lesser degree sodium. Somatropin induces the increased intestinal absorption of calcium and the increased renal tubular reabsorption of phosphorus with increased serum and inorganic phosphate. Increased serum alkaline phosphatase may also be observed during somatropin therapy.

Indications and Usage: Growth failure due to a deficiency of pituitary growth hormone is the only indication for ASELLACRIN® administration. The criteria for treatment are as follows:

1. Other causes for growth failure should be eliminated. Disorders of the pulmonary, cardiac, gastrointestinal and central nervous system and nutritional disorders which interfere with growth should be ruled out. There should be no evidence of a specific bone or cartilage disorder such as achondroplasia or other chondrodystrophy. The patient must not have psychosocial dwarfism. Primary hypothyroidism should be eliminated by appropriate laboratory testing. An abnormality of the X-chromosome should be ruled out by a karyotype in girls whenever indicated.
2. Patients must show significant short stature and/or a retarded growth rate. Patients with congenital growth hormone deficiency should be below the third percentile for height and growing at a rate of less than 5.0 cm/year over at least one year of continuous observation by the same physician. Height should be compared to appropriate standards for age. The most suitable are those compiled by the National Center for Health Statistics. Charts based on these standards are generally available. Patients with acquired growth hormone deficiency should also have grown less than 5.0 cm/year and should have been observed continuously by the same physician for at least 12 months.
3. Skeletal maturation should be compatible with a beneficial response to therapy. Epiphyseal maturation should be incomplete. In general, the response to therapy is diminished when the bone age is advanced beyond 13 to 14 years. While this is not a contraindication to the use of ASELLACRIN®, epiphyseal maturation should be below 12 to 13 years to increase the likelihood of a beneficial response.
4. The diagnosis of pituitary growth hormone deficiency should be confirmed by objective tests of growth hormone function. There must be failure to increase the serum concentration of growth hormone above 5 to 7 ng/ml in response to two standard stimuli. The stimuli which may be used are insulin-induced hypoglycemia, an intravenous infusion of arginine, oral L-DOPA, or subcutaneous or intramuscular glucagon. Suitable modifications of such procedures, such as pretreatment with estrogen or the administration of propranolol, may also be employed. Fasting serum growth hormone concentrations or the growth hormone response to exercise or sleep are not regarded as definitive tests for documentation of the diagnosis.
5. Tests of other pituitary hormone deficiencies should be carried out. Additional deficiencies should be recognized and treated where appropriate.

Deficiency of thyrotropin (TSH) must be treated before definitive testing for growth hormone deficiency can be performed. Patients must have been euthyroid for 4 to 8 weeks prior to testing. They must also have been observed for at least 6 months while euthyroid to determine whether the growth rate meets the criteria for treatment. Corticotropin (ACTH) deficiency should also be appropriately treated, as should deficiency of antidiuretic hormone. If indicated, gonadotropin deficiency may be treated concomitantly with ASELLACRIN® administration, but this may rapidly advance epiphyseal maturation and limit the long-term response to therapy.

Contraindications: ASELLACRIN® is ineffective, and should not be used, in patients with closed epiphyses.

ASELLACRIN® is contraindicated in the face of any progression of underlying intracranial lesion. Intracranial lesions must be inactive for 12 months prior to instituting therapy and ASELLACRIN® should be discontinued if there is evidence of recurrent activity.

Warnings: The possible appearance of hypothyroidism in the course of the disease, though unrelated to growth hormone therapy, must not go undiagnosed as this would jeopardize response to growth hormone.

In spite of rigorous requirements for the collection of pituitary glands used in the preparation of ASELLACRIN®, the risk of transmitting hepatitis cannot be excluded. The risk can be considered extremely small, as no cases have been reported.

Precautions: Because of its diabetogenic actions, which include the induction of hyperglycemia and ketosis, ASELLACRIN® should be used with caution in patients with diabetes mellitus or with a family history of diabetes mellitus. Regular urine testing for evidence of glycosuria should be carried out in all patients.

Local lipoatrophy or lipodystrophy resulting from subcutaneous administration may be avoided by rotating the injection site.

Bone age must be monitored annually during ASELLACRIN® administration especially in patients who are pubertal and/or receiving concomitant thyroid replacement therapy. Under these circumstances, epiphyseal maturation may progress rapidly to closure

Concomitant glucocorticoid therapy may inhibit the response of ASELLACRIN® and should not exceed 10–15 mg hydrocortisone equivalent/M^2 body surface area during the administration of ASELLACRIN®.

Patients with growth hormone deficiency secondary to an intracranial lesion should be examined frequently for progression or recurrence of the underlying disease process.

Adverse Reactions: Antibodies to somatropin are formed in 30–40% of the patients who have received somatropin prepared by similar methods. In general, these antibodies are not neutralizing and do not interfere with the response to ASELLACRIN® administration. Approximately 5% of treated patients developed neutralizing antibodies and failed to respond to somatropin. Therefore, testing for anti-somatropin antibodies should be carried out in any patient with well-documented growth hormone deficiency who fails to respond to therapy.

Dosage and Administration: Although the 2 IU and 10 IU sizes are supplied with Sodium Chloride Injection (USP) as diluent, ASELLACRIN® may be reconstituted with either Sodium Chloride Injection (USP) or Bacteriostatic Water for Injection. ASELLACRIN® may be given subcutaneous or intramuscular injection.

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Subcutaneous Injection
ASELLACRIN® may be given subcutaneously by reconstituting the 2 IU vial with 0.5 ml of diluent. When using the enclosed 2 ml ampule of diluent, discard the remaining 1.5 ml of diluent. The reconstituted vial of ASELLACRIN® for subcutaneous injection contains 2 IU somatropin per 0.5 ml, 2 IU per vial.
For subcutaneous injection, reconstitute the 10 IU vial of ASELLACRIN® with 2.5 ml of diluent. When using the enclosed 10 ml vial of diluent, discard the remaining 7.5 ml of diluent. The reconstituted vial of ASELLACRIN® for subcutaneous injection contains 2 IU somatropin per 0.5 ml, 10 IU per vial.
Intramuscular Injection
ASELLACRIN® may be given intramuscularly by reconstituting the 2 IU vial with 1 ml of diluent. When using the enclosed 2 ml ampule of diluent, discard the remaining 1 ml of diluent. The reconstituted vial of ASELLACRIN® for intramuscular injection contains 2 IU somatropin per ml, 2 IU per vial.
For intramuscular injection, reconstitute the 10 IU vial of ASELLACRIN® with 5 ml of diluent. When using the enclosed 10 ml vial of diluent, discard the remaining 5 ml of diluent. The reconstituted vial of ASELLACRIN® for intramuscular injection contains 2 IU somatropin per ml, 10 IU per vial.
It is recommended that ASELLACRIN® be administered subcutaneously or intramuscularly at a dose of 0.06 to 0.10 IU/kg three times a week. The minimum dose should be 2 IU and the maximum dose should be 5 IU three times a week. At least 48 hours should elapse between injections.
If at any time during the continuous ASELLACRIN® administration the growth rate does not exceed 2.5 cm (1 in) in a 6-month period the dose may be doubled for the next 6 months. This may be done with or without the presence of antibodies to ASELLACRIN®. If there is still no satisfactory response, ASELLACRIN® should be discontinued and the patient reinvestigated.
Treatment should be discontinued when the patient has reached a satisfactory adult height, when the epiphyses have fused, or when the patient ceases to respond to ASELLACRIN® administration.
Storage: Unreconstituted vials of ASELLACRIN® may be stored at room temperature (15°-30°C/59°-86°F).
Reconstituted vials must be refrigerated (2°-8°C/36°-46°F) and used within one month.
How Supplied: ASELLACRIN® is supplied in a sterile, lyophilized form in vials containing 2 IU or 10 IU somatropin. The following package combinations are available:
- 1 vial 2 IU ASELLACRIN® and 1 ampule 2 ml Sodium Chloride Injection (USP), NDC 44087-3002-1
- 1 vial 10 IU ASELLACRIN® and 1 vial 10 ml Sodium Chloride Injection (USP), NDC 44087-3010-1

PERGONAL®

℞

[per'go-nal]
(menotropins U.S.P.)

Description: Pergonal® (menotropins) is a purified preparation of gonadotropins extracted from the urine of postmenopausal women. Each ampule of Pergonal® contains 75 I.U. of follicle-stimulating hormone (FSH) activity and 75 I.U. of luteinizing hormone (LH) activity plus 10 mg lactose in a sterile, lyophilized form.
Pergonal® is biologically standardized for FSH and LH (ICSH) gonadotropin activities in terms of the Second International Reference Preparation for Human Menopausal Gonadotropins established in September, 1964, by the Expert Committee on Biological Standards of the World Health Organization.
Actions:
WOMEN:
Pergonal® administered for nine to twelve days produces ovarian follicular growth in women who do not have primary ovarian failure. Treatment with Pergonal® in most instances results only in follicular growth and maturation. In order to effect ovulation, hCG (human chorionic gonadotropin) must be given following the administration of Pergonal® when clinical assessment of the patient indicates that sufficient follicular maturation has occurred.
MEN:
Pergonal® administered concomitantly with human chorionic gonadotropin (hCG) for at least three months induces spermatogenesis in men with primary or secondary pituitary hypofunction who have achieved adequate masculinization with prior hCG therapy.
Indications:
WOMEN:
Pergonal® and human chorionic gonadotropin (hCG) given in a sequential manner are indicated for the induction of ovulation and pregnancy in the anovulatory infertile patient, in whom the cause of anovulation is functional and is not due to primary ovarian failure.
MEN:
Pergonal® with concomitant hCG is indicated for the stimulation of spermatogenesis in men who have primary or secondary hypogonadotropic hypogonadism.
Pergonal® with concomitant hCG has proven effective in inducing spermatogenesis in men with primary hypogonadotropic hypogonadism due to a congenital factor or prepubertal hypophysectomy and in men with secondary hypogonadotropic hypogonadism due to hypophysectomy, craniopharyngioma, cerebral aneurysm or chromophobe adenoma.
Selection of Patients:
WOMEN:
1. Before treatment with Pergonal® is instituted, a thorough gynecologic and endocrinologic evaluation must be performed. This should include a hysterosalpingogram (to rule out uterine and tubal pathology) and documentation of anovulation by means of basal body temperature, serial vaginal smears, examination of cervical mucus, determination of urinary pregnanediol and endometrial biopsy.
2. Primary ovarian failure should be excluded by the determination of gonadotropin levels
3. Careful examination should be made to rule out the presence of an early pregnancy.
4. Patients in late reproductive life have a greater predilection to endometrial carcinoma as well as a higher incidence of anovulatory disorders. Cervical dilation and curettage should always be done for diagnosis before starting Pergonal® (menotropins) therapy in such patients
5. Evaluation of the husband's fertility potential should be included in the workup.

MEN:
Patient selection should be made based on a documented lack of pituitary function. Prior to hormonal therapy, these patients will have low testosterone levels and low or absent gonadotropin levels. Patients with primary hypogonadotropic hypogonadism will have a subnormal development of masculinization, and those with secondary hypogonadotropic hypogonadism will have decreased masculinization.
Contraindications:
WOMEN:
1. A high gonadotropin level indicating primary ovarian failure.
2. The presence of overt thyroid and adrenal dysfunction.
3. An organic intracranial lesion such as a pituitary tumor.
4. The presence of any cause of infertility other than anovulation, as stated in the indications.
5. In patients with abnormal bleeding of undetermined origin.
6. In patients with ovarian cysts or enlargement not due to polycystic ovary syndrome.
7. Pregnancy.

MEN:
1. Normal gonadotropin levels indicating normal pituitary function.
2. Elevated gonadotropin levels indicating primary testicular failure.
3. Infertility disorders other than hypogonadotropic hypogonadism.

Warnings: Pergonal® is a drug that should only be used by physicians who are thoroughly familiar with infertility problems. It is a potent gonadotropic substance capable of causing mild to severe adverse reactions in women. In female patients it must be used with a great deal of care.
Precautions:
WOMEN:
1. Diagnosis Prior to Therapy
Careful attention should be given to diagnosis in candidates for Pergonal® therapy. (See sections headed "Indications" and "Selection of Patients").
2. Overstimulation of the Ovary During Pergonal® Therapy
In order to minimize the hazard associated with the occasional abnormal ovarian enlargement associated with Pergonal® -hCG therapy, the lowest dose consistent with expectation of good results should be used.
Mild to moderate uncomplicated ovarian enlargement which may be accompanied by abdominal distension and/or abdominal pain occurs in approximately 20% of those treated with Pergonal® and hCG, and generally regresses without treatment within two or three weeks.
The hyperstimulation syndrome characterized by sudden ovarian enlargement accompanied by ascites with or without pain and/ or pleural effusion occurs in approximately 0.4% of patients when the recommended dose is administered. In studies performed the overall incidence of the hyperstimulation syndrome was 1.3%.
If hyperstimulation occurs, treatment should be stopped and the patient hospitalized. The hyperstimulation syndrome develops rapidly over a period of three to four days and generally occurs during the two week period immediately following treatment. The phenomenon of hemoconcentration associated with fluid loss in the abdominal cavity has been seen to occur and should be thoroughly assessed in the following manner: 1) fluid intake and output, 2) weight, 3) hematocrit, 4) serum and urinary electrolytes, and 5) urine specific gravity. These determinations are to be performed daily or more often if the need arises. Treatment is primarily symptomatic and would consist primarily of bed rest, fluid and electrolyte replacement and analgesics if needed. The ascitic fluid should never be removed because of the potential danger of injury to the ovary.
Hemoperitoneum may occur from ruptured ovarian cysts. This is usually the result of pelvic examination. If this does occur, and if bleeding becomes such that surgery is required, the conservative approach with partial resection of the enlarged ovary or ovaries is generally adequate.
Intercourse should be prohibited in those patients in whom significant ovarian enlargement occurs after ovulation because of the danger of hemoperitoneum resulting from ruptured ovarian cysts.
3. Arterial Thromboembolism
Arterial thromboembolism following Pergonal® (menotropins) and hCG therapy has been reported in two patients, one of whom died (1).
4. Multiple Births
Of the pregnancies following therapy with Pergonal® and hCG, 80% have resulted in single births and 20% in multiple births, most of which have been twins. Fifteen percent of the total pregnancies resulted in twins, of which 93% were viable (78 surviving infants from 43 sets of twins). Five per cent of the total pregnancies have resulted in three or more conceptuses, of which only 20% were viable (nine surviving infants from three sets of triplets, four surviving infants from four sets of quadruplets, and no surviving infants from four sets of quintuplets). The patient and her husband should be advised of the frequency and potential hazards of multiple pregnancy before starting treatment.

Continued on next page

PDR®

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RECENT PROGRESS IN HORMONE RESEARCH, VOL. 54

Glycoprotein Hormone Structure-Function and Analog Design

IRVING BOIME AND DAVID BEN-MENAHEM

Department of Molecular Biology and Pharmacology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110

ABSTRACT

Human chorionic gonadotropin (hCG), luteinizing hormone, follicle-stimulating hormone (FSH), and thyrotropin (TSH) are hormones that share a common α subunit but differ in their β subunits. Recombinant DNA techniques, valuable tools for structure-function analyses, provide an approach for designing therapeutic analogs. FSH is used clinically to stimulate the ovarian follicles for *in vitro* fertilization and to initiate follicular maturation in women with infertility problems. The CGβ subunit contains a carboxy-terminal extension (CTP) with four serine O-linked oligosaccharides, which is important for the long half-life of hCG. A clinical problem of FSH is its relatively short half-life in circulation. Fusing CTP to the FSHβ coding sequence increased the *in vivo* potency of the resulting FSH dimer over three-fold. Analogs of the other hormones containing CTP also increase their biologic half-life.

Subunit assembly is vital to the function of these hormones. To address whether α and β subunits can be synthesized as one chain and also maintain biological activity, a chimera comprised of the hCG β subunit genetically fused to the α subunit was constructed. The resulting polypeptide was efficiently secreted and displayed an increased biologic activity *in vitro* and *in vivo*. Similarly, the single-chain form of FSH also retained *in vivo* activity. Since subunit dissociation inactivates the activity of the heterodimer, single-chain analogs should have longer biological half-lives. These analogs represent suitable substrates for engineering potent and stable agonists and antagonists.

I. Introduction

The family of glycoprotein hormones includes pituitary thyrotropin (TSH), lutropin (LH), follitropin (FSH), and the placental protein, chorionic gonadotropin (CG). Each hormone is a heterodimer of two noncovalently associated subunits, α and β, which are encoded by separate genes located on different chromosomes. The α subunit is common to all four hormones, while the β subunit confers the unique biological specificity for each hormone. Both subunits are glycosylated and contain asparagine (N) and, in the case of the CGβ subunit, serine-linked oligosaccharides. Their biological activity depends on the presence of intact dimers; free subunits are inactive. Newly synthesized α and β subunits are rapidly assembled in the endoplasmic reticulum and the oligosaccharides in the dimers undergo hormone-specific post-translational modifications. Thus, expression of biological activity of these hormones can be regulated at several steps in the

biosynthetic/secretory pathway. This chapter will discuss some key structure-function determinants of the glycoprotein hormone family and how expressing gonadotropins in heterologous cells can generate potentially clinically useful analogs.

II. Structure of Glycoprotein Hormone Subunits

A. THE α SUBUNIT

This subunit is a 92 amino acid polypeptide with two N-linked oligosaccharides attached to Asn 52 and 78 (Figure 1). The α subunit within a species is identical for all hormones and a product of a single gene (Fiddes and Goodman, 1981; Boothby *et al.*, 1981; Stewart *et al.*, 1987). The subunit contains 10 cysteine residues that maintain its structural integrity by forming five disulfide bonds (Mise and Bahl, 1980). The subunit combines with each of the four β subunits within an animal species; the resulting dimers express the specificity of the β subunit (Pierce and Parsons, 1981; Strickland and Puett, 1981).

B. THE HORMONE-SPECIFIC β SUBUNIT

All β subunits contain 12 cysteine residues in conserved positions and, although they determine the biological specificity of each hormone, there is a high

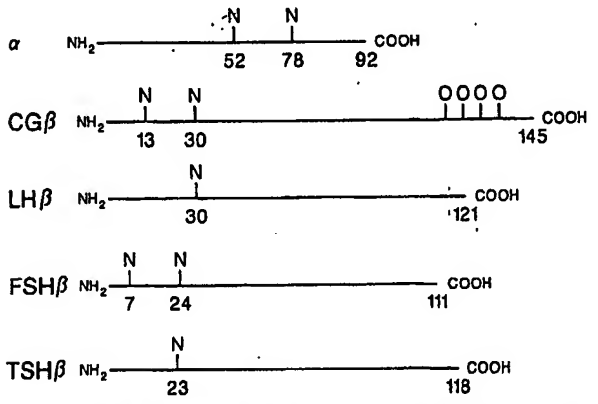


FIG. 1. Positions of asparagine-linked carbohydrate (N) in the α and β subunits of the human glycoprotein hormone family. The O designation in the CGβ subunit corresponds to the serine-linked oligosaccharides in the carboxy terminal region.

degree of sequence similarity between them (Pierce and Parsons, 1981). This is most apparent for the LHβ and CGβ subunits, which share 85% sequence identity in the first 114 amino acids. This relationship is responsible for the binding of CG and LH to a common gonadal receptor. Each of the glycoprotein hormone β subunits is a product of a different gene. A single gene encodes the LHβ (Boorstein *et al.*, 1982; Policastro *et al.*, 1986; Talmadge *et al.*, 1983), FSHβ (Keene *et al.*, 1989; Watkins *et al.*, 1987), and TSHβ (Hayashizaki *et al.*, 1985; Whitfield *et al.*, 1986) subunits, whereas the CGβ subunit is encoded by a multigene family (Boorstein *et al.*, 1982; Policastro *et al.*, 1986; Talmadge *et al.*, 1983). The length of the β subunit polypeptides varies from 111 amino acids for the FSHβ subunit to 145 amino acids for the CGβ subunit (Figure 1).

There is one N-linked oligosaccharide at Asn 30 of the LHβ and at Asn 23 of the TSHβ subunit (Figure 1). The CGβ and the FSHβ subunits contain two N-linked sugars at Asn 13 and 30, and 7 and 24, respectively (Figure 1). Compared to the LHβ subunit, the CGβ subunit contains a carboxyterminal extension of 31 amino acids (Birken and Canfield, 1977; Bousfield *et al.*, 1985), presumably due to a frameshift mutation at codon 114 in the human LH gene (Boorstein *et al.*, 1982). This carboxyterminal extension of hCGβ bears four O-linked oligosaccharides attached to serines 121, 127, 132, and 138 (Kessler *et al.*, 1979).

III. N-Linked Oligosaccharides of Glycoprotein Hormones

A. CHORIONIC GONADOTROPIN

Despite the sequence similarity of the polypeptide chains, the N-linked oligosaccharides of the glycoprotein hormones show extensive structural diversity. (The general structures are shown in Figure 2.) The predominant structures terminate with galactose and sialic acid.

B. PITUITARY GLYCOPROTEIN HORMONES

The N-linked oligosaccharides of most pituitary glycoprotein hormones from a variety of species contain terminal sulfate on their oligosaccharide units (Figure 2) (Parsons and Pierce, 1980; Green *et al.*, 1984). The majority of LH and TSH N-linked oligosaccharides are sulfated on N-acetyl galactosamine; hFSH, by contrast, terminates with galactosamine-sialic acid.

C. HORMONE-SPECIFIC OLIGOSACCHARIDE PROCESSING

From the previous discussion, it is apparent that determinants encoded in the dimer account for the hormone-specific carbohydrate structures. Subunit assembly occurs early during synthesis (Hoshina and Boime, 1982; Peters *et al.*, 1984) in the endoplasmic reticulum. The diversity of the oligosaccharide structures indicates that combination of the α subunit with a hormone-specific β subunit de-

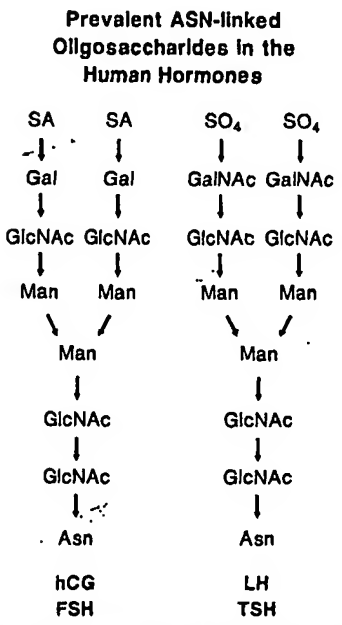


FIG. 2. Distribution of SO_4 and sialic acid (SA) in the glycoprotein hormones. GlcNAc, N-acetyl-glucosamine; GalNAc, N-acetyl-galactosamine.

termines further steps in specific processing of N-linked oligosaccharides. This results in unique oligosaccharide patterns, even if the hormones are made in the same cell and thus have access to the same set of processing enzymes (Figure 3). This is especially relevant in the case of LH and FSH: both are synthesized in the same cell and share the same α subunit and, yet, their oligosaccharides differ both in branching and terminal modification. This conclusion was supported by the results from mammalian cells transfected with gonadotropin subunit genes (Corless *et al.*, 1987). In such a system, the only variable is the β subunit introduced with the common α subunit, providing a convenient system to study the effect of subunit assembly on oligosaccharide processing. There is also, however, a tissue-specific component to maturation of the carbohydrates. Sulfation of LH

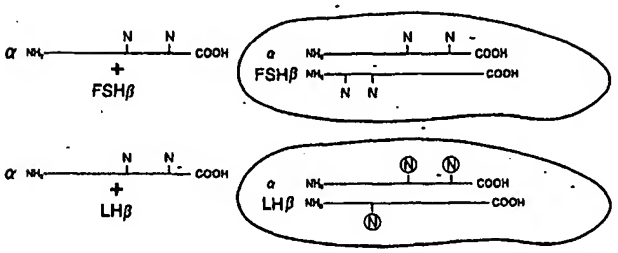


FIG. 3. Schematic diagram of co-transfection experiments with common α subunit and either LHβ or FSHβ subunit. N corresponds to the asparagine-linked oligosaccharides. The N of the FSHβ/α dimer is different from the N of the LHβ/α dimer.

and TSH requires N-acetyl-galactosamine instead of the galactose seen in sialylated chains at the penultimate position of the oligosaccharide chain. N-acetyl-galactosamine transferase is present in pituitary but not in human placenta, which also lacks the pituitary sulfotransferase (Green *et al.*, 1984; Smith and Baenziger, 1988); thus, CG oligosaccharides, in contrast to pituitary hormones, lack sulfate.

D. FUNCTION OF N-LINKED OLIGOSACCHARIDES

1. Intracellular

Oligosaccharides have been implicated in intracellular events such as folding, subunit assembly, and secretion, as well as in the biologic activity of glycoprotein hormones. To study the intracellular role of N-linked oligosaccharides in CG (Matzuk and Boime, 1988a,b), mutants of each of the four N-linked glycosylation sites were eliminated from both subunits by site-directed mutagenesis of the asparagine acceptor sites. The α and β mutant subunits were expressed separately or together to obtain CG dimer in Chinese hamster ovary (CHO) cells. The Asn 78 oligosaccharide was important for intracellular stability of the α subunit but the Asn 52 oligosaccharide was required to maintain a proper conformation for assembly. Site-directed mutagenesis also showed that the oligosaccharides in the CGβ subunit were important for proper folding and disulfide bond pairing, which is critical for efficient assembly with the α subunit (Feng *et al.*, 1995).

2. Receptor Binding and Signal Transduction

The role of the N-linked oligosaccharides in the bioactivity of the glycoprotein hormones has been studied extensively using a variety of approaches. It has

been shown that, for hCG/LH, FSH, and TSH, the oligosaccharides are not essential for receptor binding (Chen *et al.*, 1982; Sairam, 1989). The hCG and FSH oligosaccharides, especially those of the α subunit, are critical for maximal bioactivity, whereas, in the case of TSH, it has been reported that β subunit carbohydrates are important for signal transduction. It appears that oligosaccharides do not have a direct link to a signal-transducing domain in the receptor but rather maintain the proper interaction of the peptide component to the receptor-transducing domain (Feng *et al.*, 1995; Matzuk *et al.*, 1989).

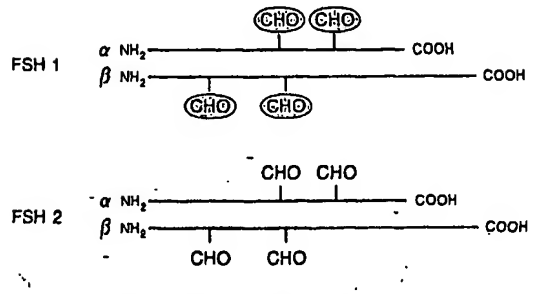
3. Half-life of Circulating Hormones

The half-life of circulating glycoprotein hormones depends on the nature of terminal modification of their N-linked oligosaccharides. Sialylation of the N-linked oligosaccharides protects the circulating glycohormones against clearance by hepatic asialoglycoprotein receptors (Morell *et al.*, 1971; Van Hall *et al.*, 1971a,b). However, sulfation leads to more-rapid clearance of pituitary hormones; sulfated bLH has a four- to five-fold greater clearance rate, compared with the sialylated recombinant bLH (Baenziger *et al.*, 1992). It was demonstrated that sulfated LH is removed from circulation upon binding to a receptor on the surface of liver endothelial cells (Fiete *et al.*, 1991). Thus, the apparent role of sulfate is to regulate the circulatory half-life of LH (Baenziger *et al.*, 1992; Fiete *et al.*, 1991; Morell *et al.*, 1971; Smith *et al.*, 1993; Van Hall *et al.*, 1971a,b). The release of LH and TSH is controlled by surges of hypothalamic-releasing hormones, GnRH and TRH, respectively (Brabant *et al.*, 1991; Clayton, 1984). A rapid-clearance system may be necessary to maintain pulsatile levels of hormones and prevent downregulation of the receptor from a sustained level of glycoprotein hormones.

Variations in the pattern of sialylation and sulfation contribute to the heterogeneity of pituitary hormones from different species analyzed by isoelectrofocusing or chromatofocusing (Figure 4). These isoforms, which differ in biopotency and circulatory half-life, are apparently under endocrine control (Chappel *et al.*, 1983; Keel and Grotjan, 1989; Matteri and Papkoff, 1988; Sergi *et al.*, 1991; Wakabayashi, 1977; Wide, 1985; Wilson *et al.*, 1990). The profile changes during development, with more-basic biologically potent species appearing at puberty (Chappel *et al.*, 1982; Ulloa-Aguirre *et al.*, 1986) and after administration of gonadotropin-releasing hormone or steroids in castrated animals (Baldwin *et al.*, 1986; Galle *et al.*, 1983). The isoforms received much attention, since regulating the final steps of N-linked oligosaccharide processing may represent a physiological way to control potency and longevity of pituitary hormones.

IV. O-Linked Oligosaccharides in the hCGβ Subunit

The CGβ subunit contains O-linked oligosaccharides that are linked to four serine residues, 121, 127, 132, and 138 (Figure 5). Studies of O-linked oligosaccharides on the hCGβ subunit were performed using recombinant DNA methods, which permitted abolishing the O-linked glycans or removing the O-linked-bearing peptide (el-Deiry *et al.*, 1989; Matzuk *et al.*, 1987,1990). The carboxyterminal O-glycosylated peptide was removed from the CGβ subunit gene by introducing a termination codon following amino acid 114 (the point at which the subunit diverges from LHβ) (Figure 6) (Matzuk *et al.*, 1990). Receptor binding and signal transduction of hCG containing the truncated β subunit studied *in vitro* remained unchanged, compared to native hormone (el-Deiry *et al.*, 1989; Matzuk *et al.*, 1990). However, truncated hCG was three-fold less potent *in vivo* than the native hormone in inducing ovulation in primed female rats (Matzuk *et al.*, 1990). Thus, as suggested earlier (Braunstein *et al.*, 1972; De Kretser *et al.*, 1973; Kalyan and Bahl, 1983; Sowers *et al.*, 1979), the O-glycosylated carboxyterminal extension of the hCGβ subunit increased the half-life of hCG in circulation.



a) Variable quantities of SO_4, Sialic acid, branching

b) Changes in overall charge affects biologic activity

FIG. 4. Variations in oligosaccharide structure and heterogeneity of a gonadotropin.

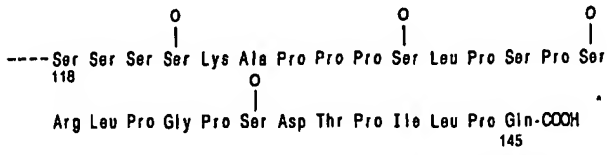


FIG. 5. The carboxy terminal extension (CTP) of the CGβ subunit. The positions of the serine-linked (O) oligosaccharides in the last 28 amino acids of the subunit are shown.

These data supported the conclusion that the presence of the sialylated O-linked sugars maintains a high level of hCG in circulation. CG is unique among human glycoprotein hormones in that its synthesis and secretion are limited to a short time window. The addition of O-linked glycans on a relatively short peptide extension may represent an adaptive response for maintaining a high level of circulating gonadotropin during pregnancy.

V. Crystallographic Analysis of CG

One of the major accomplishments in the glycoprotein hormone field has been x-ray analysis of the CG dimer (Lapthorn *et al.*, 1994; Wu *et al.*, 1994). These experiments were critical because they allowed a more-detailed definition of the subunit interactions and the conformational features of the hormone. A schematic version of the crystal structure is shown in Figure 7. One important feature revealed by this analysis is that both subunits are related to several growth factors (e.g., EGF, PDGF) that are characterized by the presence of a cystine knot. This is a cluster of disulfide bonds that apparently forms the basic scaffold of the subunit and is essential for efficient β/α combination (Ben-Menahem *et al.*, 1997). These studies also revealed a stretch of amino acids at the carboxyl end of the CGβ subunit referred as a "seat belt" that wraps around the α subunit and is presumably involved in stabilizing the heterodimer. The crystal structure also shows that the CGβ and α subunits have a remarkably similar structure (e.g., in addition to the cystine knot, they have two hairpin loops on one side of the plane and a single, larger loop on the opposite side). Future experimentation that involves crystallizing other members of the glycoprotein hormone family and ligand/receptor complexes will be invaluable for addressing numerous structure-function issues discussed here.

VI. Recombinant-derived Analogs for Clinical Use

The use of recombinant DNA technology to study structure-function biology of gonadotropins can be applied to the design of potential therapeutic agents. The

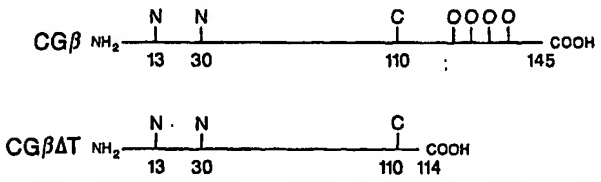


FIG. 6. A mutant hCGβ gene (CGβΔT) was generated that contained a premature termination signal at codon 115, resulting in a truncated subunit lacking the CTP sequence. This mutant was co-transfected with the α gene into CHO cells, to produce dimer.

success of such technology is now manifest in the production of recombinant hFSH. Partially purified FSH has been used to treat infertility. Until recently, such preparations were isolated from human postmenopausal urine that contained LH and other contaminating proteins. Although a variety of treatment regimens have been tried and efforts made to titrate dosages in *in vitro* fertilization protocols, multiple gestations still occur frequently. The glycoprotein hormones display significant charge heterogeneity due to structural differences in the Asn-linked carbohydrates. Since these structural isoforms display different bioactivities, species of FSH seen in commercial preparations may contribute to the complications seen with human menopausal gonadotropin administration. It would be advantageous to have a source of homogeneous FSH that could be standardized with respect to mass and bioactivity. Transfection of the FSH subunit genes into heterologous cells is a source for producing large quantities of relatively homogeneous glycoprotein hormones. This has been achieved and recombinant FSH is now available for clinical use (Olijve *et al.*, 1996).

Studies discussed above demonstrated that the dimer containing a CGβ subunit devoid of the CTP (Figure 7) sequence was three-fold less active than native CG in stimulating ovulation in rats (Matzuk *et al.*, 1990). One major issue re-

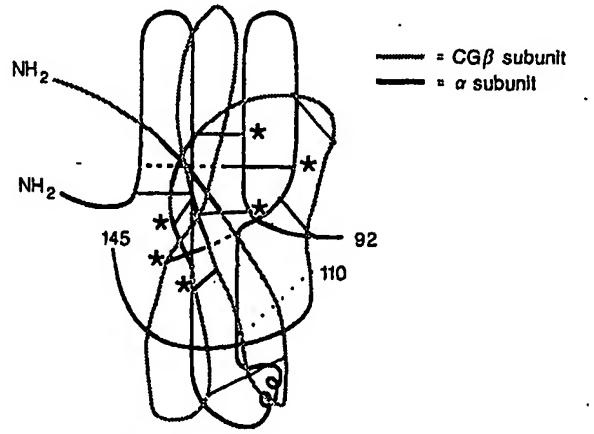


FIG. 7. Schematic diagram of the deglycosylated CG crystal structure. The asterisks indicate the disulfide bonds of the cystine knot in each subunit. The sequence 110–145 corresponds to the "seat belt" region of the CGβ subunit.

garding the clinical use of FSH is its relatively short half-life *in vivo* (Amin and Hunter, 1970; Soers *et al.*, 1979). To address this issue, the CTP of the CGβ subunit was fused to the human FSHβ subunit coding sequence (Figure 8) (Fares *et al.*, 1992). It was reasoned that this FSH analog would have a prolonged half-life and enhanced bioactivity *in vivo*, as suggested from the experiments with CG lacking the CTP sequence. These constructs were transfected into CHO cells, together with the wild-type α subunit, and stable clones were selected. Compared to wild-type FSH dimer, the addition of the CTP sequence did not significantly affect assembly, secretion, or stimulation of steroidogenesis *in vitro*. However, the *in vivo* potency increased three-fold, compared to the native hormone. Due to the relatively rapid clearance of native FSH *in vivo*, the commonly used therapeutic protocol requires frequent injections of the hormone. Thus, one immediate benefit of this analog is that the patient will require fewer injections. A similar, longer-acting TSH analog was constructed with the CTP sequence fused to the carboxyl end of the TSHβ subunit (Joshi *et al.*, 1995). Addition of the CTP could elicit immune reactions. However, several studies have demonstrated that the CTP region of CGβ is weakly immunogenic (Birken *et al.*, 1980; Matsuura and Chen, 1981). In addition, because the native CGβ subunit, which contains the CTP, is

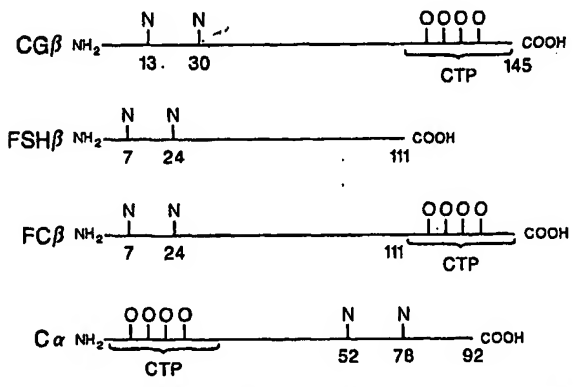


FIG. 8. Design of long-acting FSH agonist. The DNA encoding the CTP sequence (28 amino acids) was ligated to the 5' end of the FSHβ subunit gene to produce the chimera hFCβ. This construct was co-transfected with the gene encoding the common α subunit into CHO cells to generate the heterodimeric analog. Cα corresponds to an α subunit gene containing the last 28 amino acids of the CTP sequence inserted between amino acid residues 3 and 4 of the mature subunit.

normally secreted in women, the immune system may not recognize the chimera as a foreign protein.

Because the α subunit is common to the glycoprotein family, an α subunit CTP chimera could, in one construct, increase the *in vivo* stability of the entire glycoprotein hormone family. Moreover, since the α subunit sequence is dissimilar from the β subunits, the efficacy of this α subunit derivative would test the general application of CTP chimeras for increasing the biologic half-life of other bioactive proteins. Alpha subunit CTP chimeras were constructed using overlapping PCR mutagenesis (Furuhashi *et al.*, 1995). They were co-transfected with the gene encoding the hCGβ subunit into CHO cells, stable clones selected, and the *in vitro* biologic activity tested. CG dimers containing α subunit chimera with CTP at the carboxy end of the subunit had 50-fold lower binding affinity for the human LH/hCG receptor. This is presumably due to the presence of determinants for receptor binding/signal transduction at the carboxy end of the α subunit (Furuhashi *et al.*, 1995). The binding affinity of dimers composed of chimeric α subunit with the CTP inserted between amino acids 3 and 4 at the amino end of the subunit (Figure 8) was comparable to wild-type hCG. It was shown that this dimer stimulates testosterone in hypophysectomized rats, with a potency three-fold greater than wild-type hCG. These data support the usefulness of using the CTP to increase the potency of bioactive glycoproteins.

One of the characteristic features of FSH (as with the other glycoprotein hormones) is the extensive micro-heterogeneity of the hormone, caused by differences in N-linked carbohydrates. These different molecular species (often referred to as *isohormones*) are generally detected by isoelectric focusing. The more-acidic isohormones, characterized by their increased content of sialic acid, have a longer circulatory half-life than the less-sialylated or basic isohormones. Thus, the net *in vivo* stability of FSH preparation—whether recombinant or urinary derived—aptly reflects the isohormone profile in the population. It is evident that these are "natural" molecules and thus it is conceivable that, by preparative isoelectrofocusing, long- and short-acting FSH molecules can be obtained without the need for mutagenesis to alter the protein structure. One of the major issues concerning the use of recombinant FSH in assisted reproduction protocols is the potential for ovarian hyperstimulation (e.g., multiple births). Therefore, for certain regimens, it might be advantageous to have a short-acting FSH as a component of the endocrine repertory. Another approach to generate short-acting agents is to mutate the protein structure to delete one or more of the N-linked carbohydrate units (Figure 9) (Galway *et al.*, 1990).

VII. Novel Gonadotropin Model for Analog Design

It is clear that subunit assembly is vital to the function of these hormones because 1) only the dimers are bioactive, 2) maturation of the hormone-specific

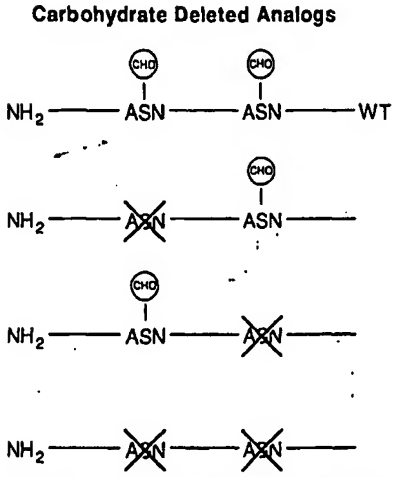


FIG. 9. Deletion of one or both oligosaccharide acceptor sites from an α and/or β subunit to shorten the *in vivo* half-life of the corresponding dimers.

oligosaccharides depends on the formation of the heterodimer complex, and 3) the secretion efficiency of the dimer is determined by the β subunit. Not surprisingly, structure-function studies of these ligands often are hampered by mutagenesis-induced defects in subunit combination and secretion of the dimer. The ability to overcome the limitations of subunit assembly/dissociation would expand the range of structure-function analyses that can be performed on these hormones. This, in turn, could lead to the engineering of analogues that have therapeutic and diagnostic applications. For example, subunit dissociation inactivates the heterodimer and, thus, single-chain analogs would be predicted to have a longer biologic half-life.

The β and α subunits were genetically linked to form single-chain derivatives (Figure 10) (Sugahara *et al.*, 1995). The consequence of this manipulation is that expressing the heterodimer as a single chain bypasses the rate-limiting assembly step. The construction of the tethered form was based on studies showing that the amino half of the β subunit and the carboxy end of the α subunit are important for receptor binding. For example, peptide addition to the carboxy end of the α subunit reduces receptor binding of the resulting heterodimer over 50-fold

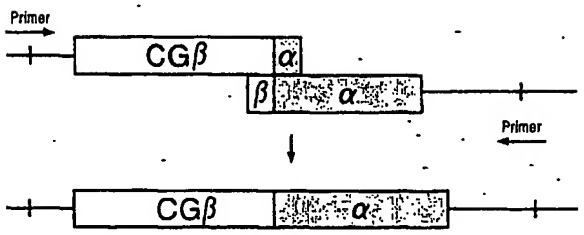


FIG. 10. Use of overlap polymerase chain reaction (PCR) to construct a single-chain gonadotropin. The gene encoding the fused gonadotropin is inserted into an expression vector and transfected into CHO cells.

(Furuhashi *et al.*, 1995). Therefore, the single chain was genetically engineered to contain the amino terminus of the α subunit without its signal peptide fused to the carboxy end of the CGβ subunit. Expression in CHO cells revealed that the secretion of the single chain and its biological activity *in vitro* were comparable to the heterodimer. These results were confirmed when the single-chain hCG was successfully expressed in insect cells (Narayan *et al.*, 1995). In the case of the CGβα tether, although no "artificial" linker was inserted to bridge the subunits, the endogenous carboxy-terminal amino acids of the CGβ subunit were exploited as a spacer (Figure 11). Because the CTP contains several proline and serine residues, it lacks significant secondary structure, is flexible and hydrophilic, and presumably would permit the α subunit to assume the proper functional orientation with respect to the β subunit domains.

Furthermore, since the CTP has determinants that increase extracellular stability of the heterodimers but does not play a role in receptor binding, the sequence could be an attractive universal linker for other multimeric proteins where increasing the biologic half-life would be desirable.

The FSH single chain also was constructed but, in this case, 29 amino acids of the CTP sequence were inserted between the FSHβ and the α subunits. The FSH single chain has a receptor-binding affinity/signal transduction activity similar to that of the corresponding heterodimer. The single chains of LH (Garcia-Campayo *et al.*, 1997) and TSH (Grossmann *et al.*, 1997) were also biologically active.

A. *IN VITRO* STABILITY OF TETHERED CONSTRUCTS

Pituitary-derived LH, FSH, and TSH are relatively unstable in aqueous solutions, compared to hCG. Because dissociation is not an issue for the single

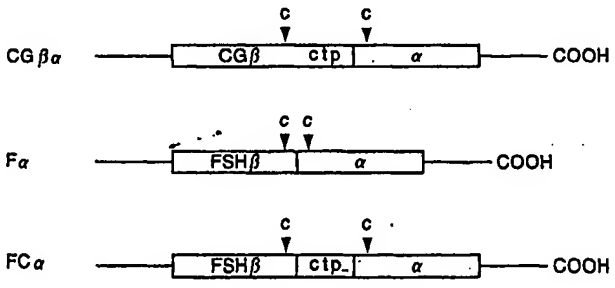


FIG. 11. Configuration of β and α domains of tethers constructed as described in Figure 10. CGβα represents the CG single chain. Fα and FCα correspond to tethers without or with the 28 amino acid stretch of the CTP sequence of the CGβ subunit. The C denotes the last carboxyl cystine residue in a β subunit and the first cystine residue at the amino end of the α subunit. These correspond to amino acids 110 and 104 of the CGβ and FSHβ subunits, respectively, and residue 7 in the α subunit.

chains, it was suspected that these glycoprotein hormone variants would be more stable *in vitro* than the corresponding heterodimers. When an aqueous solution containing single-chain LH was incubated for 2 hours at 65°C, no effect on the biologic activity was observed, while the heterodimer, incubated under the same conditions, was completely inactivated (Garcia-Campayo *et al.*, 1997).

Another approach to single-chain construction involves linking the two subunits via an intersubunit disulfide bond that is engineered by introducing pairs of cystine residues in the α and CG, LH, and FSHβ subunits (Heikoop *et al.*, 1997a). The rationale for the design of such mutants is the enhanced stability of proteins when additional disulfide bonds are introduced. These mutants were biologically active and exhibited enhanced thermostability, compared to the native heterodimers.

The single-chain approach offers a powerful tool for structure-function studies of multimeric hormones and growth factors. A single chain could have a longer *in vivo* half-life because dissociation of the heterodimer to its subunits, which are inactive, would be prevented. In agreement with this idea, it has been shown that the single-chain forms of hCG (Sugahara *et al.*, 1995) and TSH (Grossmann *et al.*, 1997) have a greater biological potency *in vivo* than the corresponding heterodimer. In addition to enhancing *in vivo* potency and *in vitro* stability, single chains provide a model to identify determinants that govern sorting to secretion pathways and formation of hormone-specific oligosaccharides. Mapping functional epitopes for intracellular and extracellular action will enable the hormone to be dissected into its active determinants.

Several studies using the single-chain model have shown that a tight association between the α and β domains is not required for biologic activity (Ben-Menahem *et al.*, 1997; Sato *et al.*, 1997). This implies that the essential role for the heterodimeric structure is to configure the common α/β determinants at the receptor/ligand interface and that bioactivity is achieved simply via independent sets of small arrays of amino acid residues in each subunit to form the contact sites with the receptor. The α/β domains can exist in different conformations, which suggests that the receptor can recognize these different forms of the ligand. That the structural features created by the α/β association are critical for receptor activation has important biological implications for producing these recombinant hormones for clinical use, since it may not always be necessary to ensure that the product has the exact native conformation. This leads to a permissiveness in making biologically active analogs. For example, regions in the single chain that are "expendable" could be identified and, as has been suggested (Heikoop *et al.*, 1997b), "mini" forms of the glycoprotein hormones can be constructed analogous to the shrinking of the atrial naturetic peptide (Cunningham and Wells, 1997). This modular design of small domains has the potential to create a new generation of clinically active agents that can be administered in different dosage forms, obviating the need for current parenteral injection protocols.

Using this model, it was shown that the major role of the cystine knot motif is to configure monomers for assembly competence (Ben-Menahem *et al.*, 1997). The biologically active form of all proteins known to have a cystine knot motif is either a homo- or a heterodimer (Sun and Davies, 1995). This capacity to dimerize is critical for the glycoprotein hormones, where the common α subunit is shared by four hormones. Arakawa *et al.* (1994) have shown that the subunits of brain-derived neurotrophic factor and neurotrophin-3, members of the cystine knot family that exist as homodimers, could assemble as biologically active heterodimers. Thus, it appears that the cystine knot is a major epitope for assembly of protein chains into their respective biologically active complexes.

In summary, the complexity of the gonadotropin structure has been a reservoir for examining a variety of fascinating structure-function concepts. The use of recombinant DNA has not only increased our understanding of glycoprotein hormone biology but investigating these issues has led to the generation of recombinant FSH as replacement therapy. A new generation of agonists (and antagonists) should be available for treating a variety of reproductive disorders.

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DISCUSSION

P. Michael Conn: Could you comment more about sulfation? Are there secretory proteins that are not biologically active? How common is this substitution? What is the role of this post-translational modification?

Irving Boime: There are numerous secretory proteins that are sulfated. A major distinction for them is whether the sulfate is linked to protein (via tyrosine) or to the carbohydrate, as in the case of glycoprotein hormones. An example of tyrosine-linked sulfate is cholecystokinin, which requires this modification for activity. In the case of sulfated glycoprotein hormones such as luteinizing hormone, sulfate is linked to a terminal N-acetyl-galactosamine of the asparagine-linked carbohydrates. This sulfated structure is recognized by receptor in the liver that is responsible for a more-rapid clearance of these hormones, compared to the sialyated structures. It has been proposed that, in the case of LH, such a rapid clearance, coupled with pulsatility, avoids desensitization.

Patrick Casey: The conclusion that multiple conformations of the hormone can interact with the receptor in a fashion that elicits full responses is quite surprising. I'm wondering if you have considered the possibility that some sort of induced-fit phenomenon was occurring upon binding? And that, if one could make constrained conformations, they would exhibit a broad range of activities?

Irving Boime: It is evident that receptor recognition does not require an intact quaternary relationship between the alpha/beta domains. We also cannot exclude a model whereby the hormone exists in several conformations and the receptor favors only the binding-competent species. We consider this less likely because no signal indicative of the native form was seen when several analogs bearing disulfide bond mutations were probed with monoclonal antibodies.

Jerome F. Strauss III: Do the mutations that you have introduced into the CG single-chain molecule cystine residues result in changes in specificity at the molecule for the glycoprotein hormone receptor family?

Irving Boime: Although we have not done a systematic analysis of all the mutants, we tested one of the CG mutants on the FSH bioassay and no activity was seen.

William J. Bremner: Despite over 60 years of research in gonadotropin biology, an active debate continues concerning the physiological role of FSH in the male. One way of experimentally approaching this issue would be to use FSH antagonists. Could you bring us up to date on what is happening in the area of developing new FSH antagonists for physiological studies?

Irving Boime: I am unaware of any significant progress in this area with respect to the ligand. There have been many mutants of FSH (and hCG) constructed, with the result that no dramatic uncoupling of receptor building from signal transduction has been seen. Possible exceptions are analogs devoid of asparagine-linked carbohydrates, which display normal receptor binding but reduced efficacy.

William W. Chin: I presume that the biological activity of CTP is largely dependent on the O-linked sugars. What, if any, is the role of the precise peptide sequence and structure? What additional evidence, other than immunologic data, do you have that the quaternary structure of single-chain hCG is not very different from the dimeric hormone? Does altering the "linker" in the single-chain hormone have any effect? Are these novel peptides immunogenic?

Irving Boime: This issue has not been resolved. While it is assumed that O-linked carbohydrates are the major players in the increased extracellular half-life of the FSH-CTP chimera (and hCG), we cannot exclude an influence on the peptide portion *per se*. This is even more provocative when we consider the recently published findings that the CTP seems to interact with the interior of the CG beta subunit; it is not simply an unstructured determinant "waving in the wind." We have no additional structural evidence other than monoclonal antibody screening, although additional physical-chemical experiments such as circular dichroism are planned. The presence of the linker is essential for maximal secretion but not for receptor binding/signal transduction. Moreover, the immunoreactivity of such linker-less molecules to monoclonal antibodies is reduced. These results are consistent with the notion of the uncoupling of the *in vivo* quaternary relationships of the alpha/beta domains from receptor binding. We have not evaluated *in vivo* the antigenicity of the single chains.

Martha Gillette: Please speculate on the relative roles of the different chains in the molecules, since it appears that one chain can activate signaling.

Irving Boime: Both the alpha and beta domains are required for receptor binding and signal transduction.

Robert Jaffe: You gave a fascinating presentation. This is a type of pituitary tumor variously called "nonsecreting" or "Ad II cell" or "glycoprotein-producing" tumor. They can often respond to noncognate hypothalamic secretagogues; that is, GnRH can sometimes stimulate TSH and TSH can sometimes stimulate FSH or LH as well as isolated subunits. What is known about the nature of the glycoprotein hormones produced by these tumors?

Irving Boime: I don't know what information is available regarding gonadotropin synthesis in this tumor but it is an intriguing model for studies of the secretagogue-coupled synthesis/secretion of LH/FSH.

CG

Development of Biopharmaceutical Parenteral Dosage Forms

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